

MUSCLING SELECTION IN SWINE AND ITS EFFECTS  
ON MUSCLE FIBER TYPES AND FIBER DIAMETER

by

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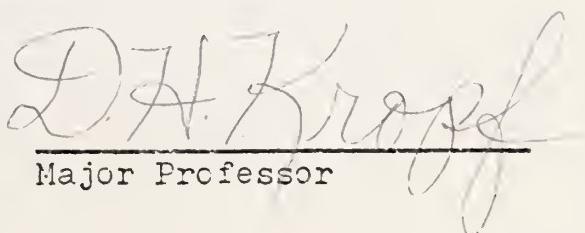
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## ORGANIZATION OF THE THESIS

This thesis is presented in a series of chapters. An introduction is presented in Chapter I. Chapter II includes a thorough review of literature.

Chapter III deals with the total study. This chapter is written in the form of a paper to be submitted to the Journal of Animal Science.

## CHAPTER I

### INTRODUCTION

Selection of heavily muscled animals for breeding stock may increase carcass "quantity" but may also decrease carcass "quality" by increasing pale, soft, exudative (PSE) or dark, firm, dry (DFD) pork (Topel, 1969; Barton, 1972).

Reports have shown that, in general, the white ( $\alpha W$ ) or intermediate ( $\alpha R$ ) fibers of porcine muscle are related to the occurrence of the PSE condition (Briskey, 1964; Cooper et al. 1969; Dilley et al. 1970; Merkel, 1971). PSE muscle showed increased cooking loss (Carpenter, 1961; Kauffman et al. 1964; Hedrick and Kauffman, 1972), reduced tenderness (Sayre et al. 1964; Kauffman et al. 1964) and increased processing losses (Kauffman et al. 1964). However, Judge et al. (1958, 1960); Hedrick et al. (1963) and Merkel (1971) reported that firmer and darker pork chops were less tender than PSE or normal pork chops.

Staun (1963) reported that animals possessing large muscle fibers are often rapid growing and more muscular. Muscle fibers with large diameters were associated with decreased tenderness (Locker, 1960; Cassens, 1966; Herring, 1968).

Low pork quality may become an industry problem and there is a real need for more insight concerning individual muscle response to selection for muscling and how this response

relates to stress and to carcass quality.

In this study, a "select" line of Duroc hogs (selection was based on an giving equal emphasis to larger loin-eye area and less backfat determined by An/Scan (sonoray)) was developed. A "control" line obtained by random selection from the same base population was also maintained. The objectives of this study were to study the muscle fiber types and fiber diameter of longissimus (L) and red semitendinosus (RST) muscles in both "select" and "control" line hogs in the fourth and fifth generations after selection was initiated. The study also included an investigation of the correlations among percentages of fiber types and fiber diameter, and of these traits to Warner-Bratzler shear force, carcass quality scores (loin color, loin marbling, loin firmness, ham color, ham marbling, ham firmness), loin-eye area, chemical analyses of longissimus (% ether extract, % moisture), and cooking losses (% total cooking loss, % drip cooking loss and % volatile cooking loss).

## CHAPTER II

## GENERAL LITERATURE REVIEW

## Classification of Muscle Fiber Types

Nomenclature. The muscle fiber is the basic unit of skeletal muscle, and comprises 75 to 90% of the total muscle mass (Hegarty 1971).

Numerous systems of classifying muscle fiber types are summarized below.

TABLE 1. SYSTEMS OF CLASSIFYING MUSCLE FIBER TYPES

	$\alpha$ W	$\alpha$ R	$\beta$ R
Ashmore and Doerr (1971a)			
Padykula and Gauthier (1967)	White	Intermediate	Red
Ogata (1958b)	White	Intermediate	Red
Samaha et al. (1970)			
Yellin and Guth (1970)	$\alpha$	$\alpha\beta$	$\beta$
Stein and Padykula (1962)	A	C	B
Romanul (1964)	I	II	III
Brooke and Kaiser (1970)	IIB	IIA	I
Engel (1962)	II	II	I
Burke et al. (1971)	FF	FR	S
	(Fast contracting fast fatigue)	(Fast contracting fatigue resistant)	(Slow contracting)
Barnard et al. (1971)	Fast-twitch white	Slow-twitch intermediate	Fast-twitch red
Beecher et al. (1965a)	Light	Dark	Dark
Peter et al. (1972)	Fast-twitch glycolytic	Fast-twitch oxidative-glycolytic	Slow-twitch oxidative

All systems mentioned above for classification of muscle fibers are currently in use. Therefore, considerable confusion is possible.

Muscle fiber classification. Muscle fiber types have been classified into two groups, dark and white (Nachmias and Padykula, 1958; Engel, 1962; Beecher *et al.* 1965a), four groups (Brooke *et al.* 1970) or even eight groups (Romanul, 1964).

Ogata (1958b) classified the muscle fiber types as red, intermediate and white. Stein and Padykula (1962) classified the muscle fiber types into A, B and C based on the succinate dehydrogenase (SDH) staining.

Engel (1962) had designated muscle fibers as Type I and Type II as differentiated by SDH staining and Brooke and Kaiser (1970) designated fibers as IIB, I and IIA based on adenosine triphosphatase (ATPase) staining.

Padykula and Gauthier (1967) used differences in mitochondrial enzyme activity between fibers to distinguish among red, white and intermediate. More recently, Samaha *et al.* (1970) and Yellin and Guth (1970), demonstrated three fiber types based on ATPase staining. The three fiber types were designated  $\alpha$  (white),  $\beta$  (red) and  $\alpha\beta$  (intermediate). Ashmore and Doerr (1971a,b, 1972b, 1974) studied chicken, porcine, bovine and ovine muscles and reported two basic muscle fiber types ( $\alpha$  and  $\beta$ ) based on myosin ATPase activity.

Peter *et al.* (1972) referred to fibers as slow-twitch oxidative, fast-twitch oxidative glycolytic and fast twitch

glycolytic. Burke et al. (1971) had designated fibers as S (slow contracting), FR (fast contracting, and fatigue resistant), and FF (fast contracting, fast fatigue). This system is based on physiological features of the muscle fibers, but the cytochemical features, described by them, are the same as those described by Ashmore et al. (1972b) for  $\beta$ R,  $\alpha$ R and  $\alpha$ W fibers, respectively.

Barnard et al. (1971) studied guinea pig muscle and used the terms slow-twitch intermediate, fast-twitch red and fast-twitch white to identify fibers on the basis of a contractile characteristic and on a metabolic characteristic. However, this system was developed from studies on mice and guinea pigs and cannot be applied to most other species, since guinea pig "intermediate" fibers, based on SDH activity, are fast fibers not slow fibers (Ashmore et al. 1971a,b).

Some investigators have been using the term "intermediate" to classify different fiber types in different species. Ashmore et al. (1971b) stated that the intermediate fiber in mouse, rat and guinea pig muscles is equivalent to the  $\beta$ R fibers, whereas in other species it is more often equivalent to the  $\alpha$ R fibers.

Cooper et al. (1970) studied "intermediate" fibers which exhibited an "intermediate" reaction to reduced nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) and a positive reaction to myosin ATPase. However, Edgerton and Simpson (1969) characterized "intermediate" fibers which

demonstrated a moderate reaction with nicotinamide adenine dinucleotide-diaphorase (NADH-D) and a minimal reaction with myosin ATPase. It is easy to see that these two "intermediate" fibers are different. Therefore, the term "intermediate" should not be used for muscle fiber classification (Ashmore *et al.* 1971b).

Ashmore and Doerr (1971a) suggested that three main types of fibers are differentiated by cytochemical analysis:

- a; Slowly contracting fibers with aerobic metabolism ( $\beta R$ )
- b; Rapidly contracting fibers with aerobic metabolism ( $\alpha R$ ) and
- c; Rapidly contracting fibers with anaerobic metabolism ( $\alpha W$ ).

#### Red and White Muscle

Introduction. The existence of red and white muscle has been known for some time, and Lorenzini (1678) is credited for the description of muscles of varying color intensity in the rabbit limb. Ranzier (1874) showed that redness was associated with slowness of contraction and with the genesis of tetanus at lower rates of stimulation. Denny-Brown (1929) and Ogata (1958a) reported that muscle color is dependent upon the proportion of red fibers which the muscle contains. Beecher *et al.* (1965b) reported that porcine "red muscles" contain more than 40% red fibers, and "white muscles" contain less than 30% red fibers. White muscles include semitendinosus light portion, outside biceps femoris, longissimus and gluteus medius. Red muscles include serratus ventralis, rectus femoris, inside

biceps femoris, semitendinosus red portion and trapezius.

Red muscle characteristics. Lawrie (1953) and Tapple and Martin (1958) reported that red muscles have high oxidative enzyme activity whereas white muscles have high glycolytic activity.

Beecher et al. (1965b) reported that red muscles contain greater myoglobin concentrations and generally have longer post-rigor sarcomeres than white muscles, which may be an essential feature related to their slower postmortem glycolytic rate and greater resistance to postmortem alternation. Beecher et al. (1965b) found that pH drop tends to be slower in the red muscles after 2 hours postmortem whereas SDH activity in red muscles is higher than in white muscle. Milo et al. (1964) concluded that rigor mortis developed more quickly in white than in red muscle, but their evaluations are based on in situ estimations. Forrest et al. (1966) observed no significant difference between red and white muscles in response to electrical stimulation or in the time course of rigor mortis.

White Muscle Characteristics. Ashmore et al. (1972b) indicated that rapid and extreme changes in postmortem muscle pH would be associated with white muscles. Since rate of pH decline primarily reflects capacity of muscle for anaerobic metabolism and, is in essence, the net result of both antemortem and postmortem metabolism of muscle glycogen stores.

Kowalski (1969) stated that white muscles like rectus femoris and vastus intermedius are highest in phosphorylase

activity. A rise in phosphorylase activity (due to a running exercise) was observed in muscles generally low in phosphorylase activity.

Red and White Portions of Semitendinosus. Beecher et al. (1965a) reported that the semitendinosus muscle in pigs is noted to have a decidedly red area (inside) proximal to the femur and a lighter area (outside) near the subcutaneous fat layer. They found myoglobin concentrations more than 100% higher in the darker red proximal portion. Also, the dark portion had higher SDH activity than the light portion. Hunt and Hedrick (1977a,b) indicated that bovine inside semitendinosus (red) muscle had more ether extractable constituents and less moisture than the outside semitendinosus (white) muscle, confirming that red fiber areas contain more lipid than white fiber areas (Ashmore et al. 1972b).

Franke and Solberg (1973) studied enzyme distribution in four post-rigor bovine muscles. They examined five areas within each muscle surface area and also four longitudinal locations. Fiber type did not vary longitudinally within a muscle. The semimembranosus (SM) and adductor had uniform fiber type distributions while the bicep femoris (BF) and semitendinosus (ST) fiber types varied greatly between the five sampled areas within each muscle. Why the ST and BF have red and white areas but SM and adductor are homogeneous in their fiber types is not clear.

## Characteristics of Muscle Fibers and Their Relation with Animal Growth and Muscling

Muscle fiber growth and development. Hegarty (1971) indicated that the fiber or cell is the basic unit of skeletal muscle and comprises 75 to 90% of the total muscle mass. Cassens et al. (1969) reported that the differentiation of muscle fibers during growth and development is important to meat science. Fiber type composition of adult muscle is responsible for its gross characteristics and its behavior postmortem.

Rowe and Goldspink (1969) reported that postnatal growth of muscle fibers is achieved by muscle fiber hypertrophy (increase in fiber size) through lengthening and thickening of individual fibers. Ashmore et al. (1972b) and Rowe and Goldspink (1969) stated that fiber number in a muscle is relatively fixed at birth. Increasing muscularity in domestic animals may be achieved by transformation of  $\alpha R$  fibers to  $\alpha W$  fibers.

Dubowitz (1965) studied the enzyme histochemistry of animal skeletal muscle and found muscle fibers in guinea pigs can be fully differentiated at birth. Muscle fibers from rabbits and hamsters can be partially differentiated at birth, and in the mouse and rabbit, muscle fibers are undifferentiated at birth. Muscle fibers may be differentiated at birth in more mature and mobile animals whose maturity increases as

length of gestation increases. Cooper et al. (1970) reported fiber types of postnatal pigs show no differentiation immediately after birth. Within one to three weeks, differentiation began and is very evident after four weeks of age. The percentage of white fibers increased steadily with age.

Ashmore et al. (1972b) found fewer  $\beta$ R fibers in less active porcine, ovine and bovine muscles. A small proportion of the  $\alpha$ -fiber population remains in the red state ( $\alpha$ R). Muscles which are used to maintain posture have a high proportion of red fibers ( $\alpha$ R or  $\beta$ R). Vigneron et al. (1976) examined longissimus muscle of the rabbit at four crosssectional locations from anterior to posterior. The total number of muscle fibers as well as the percentage of  $\beta$ R,  $\alpha$ R and  $\alpha$ W fiber types were estimated at each level. Significant differences were found between locations for percentages of  $\alpha$ R and  $\beta$ R fiber types, but not for the  $\alpha$ W fiber types. The total number of fibers increased fourfold from anterior to posterior. The percentage of  $\beta$ R fibers decreased from approximately 10% at the anterior end of the muscle to 3% at the posterior end, while the percentage of  $\alpha$ R fibers increased from 34 to 41%.

Reddy (1971) reported that bulls and steers do not differ in mean percent fiber types or percent fiber area. Fiber type, size and percent fiber area were not affected by energy level.

Muscle fiber transformation. Ashmore et al. (1972b) indicated red fibers ( $\alpha$ R and  $\beta$ R) tend to be located internally

in fiber bundles of adult muscle in pigs. The  $\alpha$ -fibers ( $\alpha$ W) which form from  $\beta$ -fibers during development are displaced outwardly, thus occupy more peripheral positions in the bundles.

Ashmore et al. (1971a, 1972a,b) studied chicken, porcine, bovine and ovine muscle and stated that there are two basic muscle fiber types ( $\alpha$  and  $\beta$ ) based on myosin ATPase activity. Initially,  $\alpha$  fibers are red, but have the capacity to transform from an aerobic state of metabolism ( $\alpha$ R) to an anaerobic state ( $\alpha$ W). Beta fibers remain red through the lifespan. In pigs at two weeks of age it is clear that some  $\alpha$ R fibers are beginning to transform to  $\alpha$ W fibers. Commonly,  $\alpha$ R fibers are adjacent to  $\beta$ R fibers. In chicken, nearly all  $\alpha$  fibers in the pectoralis muscle transform completely to white fibers during the first two to three weeks after hatching. Hollyoszy and Oscai (1969) indicated that it is possible that  $\alpha$  fiber transformation may, to a degree, be reversible.

Cassens et al. (1968) established, with the Sudan Black B reaction, that porcine longissimus muscle is not differentiated into red and white fibers during foetal stages. All fibers are Sudan Black B positive (red) on the day of birth, but by the thirteenth postnatal day about 60% of the fibers have differentiated to white (Sudan Black B negative). Approximately 15% of the fibers remain as red fibers when the animal reaches 200 days old.

Muscle fiber size. Ashmore et al. (1972b) indicated that

white muscle fibers ( $\alpha W$ ) are considerably larger on the average than red muscle fiber types ( $\alpha R$  and  $\beta R$ ). The size of a given muscle is going to vary directly with the proportion and degree of  $\alpha R$  fiber transformation to  $\alpha W$  fibers. A number of characteristics are summarized in Table 2.

Myoglobin, glycogen and lipid. Lawrie (1952) reported that red fibers had high myoglobin content which is associated with high SDH activity. Phospholipid content of red fibers is higher than the phospholipid content of white fibers. Beecher et al. (1968) reported that myoglobin concentration and percent red fibers were more than two-fold higher in the semitendinosus dark portion than the semitendinosus light portion.

Beatty et al. (1963) found glycogen content of white muscle (small laboratory animals) to be higher than red muscles. George and Naik (1958) found a 1 to 5 ratio of glycogen content when they compared red and white fibers, respectively, of pigeon pectoralis major muscle. Also Ogata (1960) found 3.7 times more glycogen in white compared with red muscle of rabbit. Bocek et al. (1966b) reported higher glycogen levels in rat red muscles than in white muscles. Beecher et al. (1965a,b) reported higher glycogen concentrations in the semitendinosus dark portion than in the semitendinosus light portion. But Beecher et al. (1968) reported that glycogen concentration is similar in the semitendinosus light and dark portions.

Beecher et al. (1965a) observed that trapezius (red) contains more than twice as much lipid as does longissimus

Table 2. COMPARISON OF CHARACTERISTICS AMONG  $\beta$ R,  
 $\alpha$ R AND  $\alpha$ W FIBERS.

Characteristic	$\beta$ R <sup>a</sup>	$\alpha$ R	$\alpha$ W
Diameter	Narrow	Intermediate	Broad
Color	Red	Red	White
Blood supply	High	Intermediate	Low
Capillary:fiber	High	High	Low
Myoglobin	High	High	Low
Glycogen	Low	High	High
Contraction speed	Slow	Fast	Fast
Fatigue	Slow	Slow	Fast
Sarcoplasmic reticulum	Less	Less	More
M-line	Absent	Absent	Present
Z-line	Wide, rough	Wide, rough	Narrow, smooth
Function	Postural	Postural	Rapid movement
Location in bundles	Central	Central	Peripheral
Metabolism	Aerobic	Aerobic	Anaerobic
Mitochondria	More	More	Less
Lipid	High	High	Low
Phosphorylase	Low	Intermediate	High
SDH <sup>b</sup>	High	Intermediate	Low
SDH/phosphorylase <sup>c</sup>	High	Intermediate	Low
Glycolytic enzyme	Low	Intermediate	High
Lactic dehydrogenase	Low	Intermediate	High
NADH-TR <sup>d</sup>	High	Intermediate	Low
Myofibrillar ATPase alkaline	Low	Intermediate	High
Myofibrillar ATPase acid	High	Intermediate	Low

a Classification system suggested by Ashmore and Doerr, 1971a

b SDH = Succinate dehydrogenase.

c SDH/phosphorylase is an index of aerobiosis.

d NADH-TR = Nicotinamide adenine dinucleotide-tetrazolium.

(white). Allen *et al.* (1967) had shown a greater lipid content in longissimus than in psoas muscle. Porcine longissimus muscle (white) and psoas muscle (red) were not different in lipid phosphorus, cholesterol and cholesterol esters. The fatty acid composition of the lipid extracts of longissimus had significantly more C<sub>14</sub> and C<sub>18:1</sub> and less C<sub>18:2</sub> than psoas. Lipids from red muscle are slightly less saturated than those from white muscle. Ashmore *et al.* (1972b) found red fiber types ( $\alpha R$  and  $\beta R$ ) metabolize and store more lipid than white fibers. Therefore, red and white muscle differ in quality traits.

Contraction speed. Romanul (1965) reported red fiber types ( $\alpha R$  and  $\beta R$ ), which are adapted for repetitive contraction, require continual energy production, and therefore, a constant source of oxygen, nutrients, and waste removal. These could be best satisfied by small fiber size and high capillary : fiber ratio. Barnard *et al.* (1971) studied guinea pigs and showed that  $\beta$  fibers have slower contraction speeds than  $\alpha$  fibers. Ashmore *et al.* (1971a) indicated that  $\alpha$  fibers ( $\alpha R$  and  $\alpha W$ ) are physiologically fast fibers, whereas  $\beta$  fibers ( $\beta R$ ) are slow fibers.

Mitochondria. Padykula and Gauthier (1963) described a difference in the size and shape of mitochondria in red and white muscle fibers of the rat diaphragm muscle. Dense accumulations of mitochondria were observed by electron microscopy in the small fibers but size and shape varied. Lipid droplets were often dispersed among the mitochondria. In the large fibers,

there was a general absence of large mitochondria in the periphery of the fiber. Hollyoszy and Oscai (1969) isolated mitochondria from exercised muscle. They exhibited increased capacity to oxidize palmitate, and a decreased capacity to oxidize  $\alpha$ -glycerol phosphate. These are known to be characteristics of red fiber mitochondria.

Glycolysis. Beecher et al. (1965a) and Bocek et al. (1966a) reported that glycolysis, immediately postmortem, in white muscle is accelerated to a greater degree than in the red muscle by the physiological stress at death. Beecher et al. (1968) found lactic acid concentrations are higher in the semitendinosus light portion than in the semitendinosus dark portion. Fibers which have a high capacity to produce high energy via anaerobic metabolism generally contain higher levels of glycogen, phosphorylose and phosphofructokinase, rate limiting enzymes for glycogenolysis and glycolysis (Bonilla and Schotland 1970).

Enzyme activity. Nachmias and Padykula (1958) used SDH staining and found the smaller dark fibers exhibit greater SDH activity than the larger light fibers. Fibers of intermediate size and activity are also observed. Edgerton and Simpson (1969) found in mouse, guinea pig and rat muscle,  $\beta$ R fibers exhibit an SDH reaction intermediate between that of  $\alpha$ R and  $\alpha$ W fibers. This observation has been the source of some confusion. They also stated that  $\beta$  fibers constantly exhibit the highest SDH/glycogen phosphorylase activity ratio and are adapted for aerobic

metabolism. Alpha fibers exhibit a wide range of SDH/glycogen phosphorylase ratio. Beta fibers can consistently be classified as red fibers,  $\alpha$  fibers could be classified as red, white or intermediate fibers based upon their energy-producing enzyme. Ashmore et al. (1972b) reported in species examined to date (except mouse),  $\beta$ R fibers generally exhibit equal or more intense SDH activity than  $\alpha$ R fibers. Ashmore et al. (1972b) reported that in muscles of piglets (Duroc) all muscle fibers exhibit high SDH activity. Glycogen phosphorylase activity is weak in  $\alpha$  fibers near the periphery of fiber bundles, and absent in  $\alpha$  and  $\beta$  fibers in the interior of fascicules. Glycogen phosphorylase activity is higher in newborn ovine and bovine muscles than in newborn porcine muscles. Alpha fiber transformation begins earlier in calves and lambs than in pigs.

Guth and Samaha (1969) exposed muscle tissue sections to acid and alkali prior to the ATPase reaction medium and were able to clearly demonstrate differences between fibers. Slow fiber ( $\beta$ R) lost activity during alkali preincubation, but activity persisted through acid treatment. The pattern was the reverse for fast fibers ( $\alpha$ W). They concluded at least two distinct myosin ATPases exist, one for each type of fiber. Barany et al. (1965) and Samaha et al. (1970) stated that purified myosin from slow and fast muscle differs with respect to specific ATPase activity, acid and alkali stability, susceptibility to tryptic digestion and in the number and molecular weights of light chain components.

Ashmore et al. (1971a,b) found for muscle fibers in chicken, mouse, bovine and porcine, that the  $\alpha$ -fiber population generally exhibits a wide range of activities of both aerobic and anaerobic enzymes.

Hormones. Little information is available concerning a possible role hormones may play in regulation of fiber types. Bass et al. (1971) observed that testosterone promotes transformation of  $\alpha R$  to  $\alpha W$  fibers in guinea pig temporal muscle. But it is doubtful whether any advantage could be gained by its application to meat animal species.

Close (1972) concluded that red fibers contain myoglobin and generate ATP by oxidation of fat and carbohydrates. They are generally smaller in diameter which facilitates rapid exchange of substances and toxic waste products, therefore exhibit resistance to fatigue. White fibers ( $\alpha W$ ) depend primarily upon anaerobic glycolysis to produce ATP, contain little or no myoglobin and, therefore, tire quickly during strenuous activity as endogenous glycogen stores are depleted.

Beecher (1966) concluded that red fibers ( $\alpha R$  and  $\beta R$ ) can be characterized as granular, dark, slow, small, tonic and red, while white fibers ( $\alpha W$ ) could be termed as agranular, bright, light, fast, large, pale, tetanic and twitch.

#### Muscle Fiber Types Vs Meat Quality

Hegarty (1971) reported that a muscle is composed of

70 to 90% muscle fibers, so the composition of muscles is influenced dramatically by the muscle fiber types present.

Ashmore et al. (1972b) suggested that modern selection pressure for heavy-muscled efficient farm animals has been selecting for a higher percentage of larger, anaerobic muscle fibers ( $\alpha W$ ) which may subsequently result in lower muscle quality (lower marbling), i.e. double muscling and PSE pork, since white muscles ( $\alpha W$ ) are associated with less intramuscular lipid. Rapid reduction and extreme variations in postmortem muscle pH are more likely to be associated with white muscles since the pH of meat primarily reflects capacity of muscle for anaerobic metabolism. Larger amounts of fat within muscles (intramuscular and intracellular fat) are most generally associated with muscles which have a large proportion of  $\alpha R$  and  $\beta R$  fibers. George and Bhakthan (1961) also indicated that red muscle fibers ( $\alpha R$  and  $\beta R$ ) have higher lipid concentrations. PSE pork (Dilley et al. 1970), and double-muscling in cattle (Ashmore et al. 1972b) are directly related to an increase in the proportion of white ( $\alpha W$ ) fibers. Lawrie et al. (1963) stated that hypertrophic muscle (double muscle) possesses considerably less fat and less visible fat between the fiber bundles.

Rao et al. (1968) reported that beef carcass maturity and marbling had no effect on proportion of red, white or intermediate fibers. The interaction between marbling and maturity in proportion of red fibers was significant when all

combinations of small and moderate marbling and A<sup>-</sup>A, A<sup>+</sup>B<sup>-</sup> and BB<sup>+</sup> maturity were studied. Fat, protein or moisture percentage, mean fiber diameter and muscle color reflectance at 474  $\mu$ m at zero oxygen exposure were not significantly correlated with percentage of various fiber types.

Melton et al. (1974) reported that % red fiber area was significantly related to measures of increased animal weight and degree of fat deposition but not to palatability traits. Areas of intermediate and white fibers were positively related to panel flavor score and % protein of the biopsy muscle tissue.

Morita et al. (1970a,b) found that myoglobin positive fibers are positive for oxidative enzymes. A close correspondence was noted between fiber classification (red, intermediate or white) and myoglobin staining intensity. The percentage area of myoglobin positive fibers ( $\alpha$ R and  $\beta$ R) was not strongly associated with color score of pig muscle.

Melton et al. (1975) studied the relationships between beef quality or cutability and various histochemical characteristics of biopsy longissimus muscle samples from 21 Hereford yearling bulls in five sire groups. They indicated that percentage of fiber types was not closely associated with any palatability trait except juiciness.

Taste panel juiciness score was negatively correlated ( $r=-.48$ ) with percent  $\beta$ R fibers and positively correlated ( $r=.48$ ) with percent  $\alpha$ R and  $\alpha$ W fibers.

Melton (1971) reported that taste panel juiciness was negatively correlated (-.66) with %  $\beta$ R fibers.  $\beta$ R fiber area was positively correlated with live weight (.59), external fat thickness (.75), yield grade (.67), marbling score (.49) and U.S.D.A. quality grade (.54).

Reddy (1971) indicated % Type I ( $\beta$ R) fiber was correlated with muscling score ( $r=.47$ ); steer % Type II ( $\alpha$ W) fiber with marbling ( $r=.72$ ), final grade ( $r=.71$ ), and ether extract ( $r=.74$ ). Water holding capacity (WHC) was positively correlated with area of Type I ( $\beta$ R) ( $r=.77$ ) and area of intermediate fibers ( $\alpha$ R) ( $r=.64$ ). Type II fiber ( $\alpha$ W) area was correlated with cooking loss ( $r=-.47$ ), WHC ( $r=-.49$ ) and firmness ( $r=-.72$ ).

May (1975) studied three different cattle types namely 12 Simmental X Angus (SXA), 12 Hereford X Angus (HXA) and 12 Limousin X Angus (LXA) and indicated that % fat in longissimus increases, %  $\alpha$ W fiber decreases and  $\alpha$ R and  $\beta$ R fibers increase slightly with longer feeding (200, 242 and 284 days after weaning). Alpha white fibers were related positively but not significantly to indices of muscling. Generally, over all breeds, there was no significant correlation between histological characteristics and palatability traits. The only significant correlations were obtained between tenderness and % fibers ( $\alpha$ W and  $\beta$ R) and fiber diameters of  $\alpha$ W and  $\beta$ R.

Reddy (1971) indicated that bulls and steers are not different in mean % fiber types or % fiber area. Fiber type, size and % fiber area were not different for low and high

dietary energy level.

#### Histochemical Staining Methods

Succinate dehydrogenase (SDH) staining. Seligman and Rutenburg (1951) described a reliable and sensitive SDH staining method. Padykula (1952) demonstrated a variation in SDH activity in the muscle of the rat. He categorized fibers according their metabolic functions as revealed by strong or weak reactions for SDH activity. Ogata (1958a) studied muscle SDH activity in fish, frogs, birds and mammals and recognized three muscle fiber types; a large white fiber with weak enzyme activity, a small red fiber with strong activity and a medium fiber with intermediate enzyme activity.

Barka et al. (1963) outlined the procedure used in their laboratories for histochemical demonstration of SDH activity. Romanul (1964) studied entire muscle cross-sections to learn if there was a pattern of fiber type distribution. Sections of the gastrocnemius, plantaris, soleus and heart of albino rats were used in a floating incubation, followed by a 10% formalin fixation.

The SDH method of Nachlas et al. (1957) using Nitro BT incubation medium showed oxidative activity differences between and within muscles. The gastrocnemius muscle was found to have fibers with both higher and lower SDH activity than the soleus.

SDH is an enzyme which is purely mitochondrial and which also gives good differentiation of fiber types.

Ashmore and Doerr (1971a) reported that in species examined to date, with the exception of the mouse and guinea pig,  $\beta$ R (red) fibers generally exhibit equal or more intense SDH activities than  $\alpha$ R (intermediate) fibers.

Adenosine triphosphatase (ATPase). ATPase activity was early studied by Glick and Fischer (1954). This method was not used for years, because its specificity was doubtful. In 1955, Padykula and Herman developed a faster and more sensitive ATPase method. Guth and Samaha (1970) described an actomyosin ATPase method which is a modification of Padykula and Herman's technique. Three muscle fiber types can be histochemically distinguished on the basis of a qualitative difference in their actomyosin ATPase. This ATPase of the white fiber ( $\alpha$ W) is acid-labile and base-stable, the ATPase of the red fiber ( $\beta$ R) is base-labile and acid-stable, and the intermediate ( $\alpha$ R) is intermediate in stability at both an acidic and basic pH. Preincubation media pH is very critical. The optimum pH and duration of both the acid and alkaline preincubations will depend on the muscles and the species.

Padykula et al. (1963) delineated the differences between mitochondrial ATPase and myosin ATPase. Mitochondrial ATPase was active at pH 7.2, required magnesium, was stimulated by dinitrophenol and was not sulfhydryl dependent. Myosin ATPase was active at pH 9.4, was located in the A-band, was

suppressed by p-hydroxymercuri-benzoate and therefore was sulfhydryl dependent.

Berg et al. (1972) reported a method for an accelerated histochemical ATPase reaction in the presence of chloride salt. The reaction involved magnesium and lead.

Ashmore and Doerr (1971a,b) believed that to correctly describe a fiber, it is necessary to denote its ATPase activity as well as its "metabolic character". Using myosin ATPase and SDH staining, fibers that stain darkly (positive ATPase) at alkaline and have a positive SDH are considered  $\alpha R$  (intermediate); fibers which stain darkly (positive ATPase) and have a negative SDH are considered  $\alpha W$  (white); those which stain lightly (negative ATPase) and have a positive SDH are considered  $\beta R$  (red).

Nicotinamide adenine dinucleotide - tetrazolium reductase (NADH-TR). Farber et al. (1956) were the first to histo-chemically localize diphosphopyridine nucleotide diaphorase (DPND) and triphosphopyridine nucleotide diaphorase (TPND). These are oxidative enzymes which reflect the utilization of various metabolic intermediaries of the Krebs cycle and related pathways. They, therefore, give an indication of the possible sources of energy in muscle metabolism. The dehydrogenases are substrate-specific oxidative enzymes which transfer electrons (hydrogen) from a substrate to an acceptor. With the exception of SDH and mitochondrial  $\alpha$ -glycerophosphate dehydrogenase, they all require added coenzyme, either NAD or

NADP, to act as an intermediate carrier of the electrons to molecular oxygen.

The principle of the histochemical technique is to employ a colorless, soluble, tetrazolium salt which intercepts the electrons at some point along the respiratory chain and is reduced to a deeply colored, insoluble formazan product which is deposited at the site of enzyme activity. Commonly used tetrazolium salts are 2,2'-di-p-nitro-phenyl-5,5'-diphenyl-3,3'-(3,3'-dimethoxy-4,4'-biphenylene) ditetrazolium chloride (Nitro-BT; NBT) and 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT).

The most elementary of the oxidative enzyme reactions is the NADH-TR reaction which is synonymous to NADH dehydrogenase or NADH-diaphorase or DPNH-diaphorase (in old terminology). In NADH-TR staining, the  $\alpha$ W (white) fibers are considered negative,  $\alpha$ R fibers (intermediate) are considered positive and the  $\beta$ R fibers (red) are considered essentially positive.

Pale, Soft and Exudative (PSE)  
and Dark, Firm and Dry (DFD) Pork

Occurrences. The major physiological abnormalities associated with porcine stress syndrome (PSS) were the same as those responsible for development of pale, soft, exudative (PSE) porcine muscle. The signs for the porcine stress syndrome can definitely result in the development of PSE pork

muscle but they can also result in the development of dark, firm, dry (DFD) pork muscle (Topel, 1968; Barton, 1972). The ultimate muscle characteristics are highly associated with the length and duration of stress conditions prior to slaughter. PSE and DFD muscle would be considered the same from a genetic standpoint.

Lawrie (1966) found PSE pork is the manifestation of short-term adrenaline release in muscles adapted for anaerobic activity, and dark cutting beef (higher pH value at 24 hours postmortem) is the manifestation of long-term adrenaline release in muscles adapted for aerobic activity.

The pale, soft and exudative (PSE) condition of porcine muscle had been associated with heavy muscling (Ludvigsen, 1960; Judge et al. 1968). The PSE condition occurs when a low muscle pH develops soon after exsanguination while muscle temperature remains high (Ludvigsen, 1954; Wismer-Pedersen et al. 1961b; Beecher et al. 1965a). Wismer-Pedersen (1959) stated that development of PSE musculature includes a lowered or possibly denatured muscle pigment component. McLoughlin and Goldspink (1963) stated that muscle pigment is masked by precipitated sarcoplasmic proteins so as to cause a lighter appearance of the PSE muscle surface. Cassens et al. (1963) observed from electron micrographs of PSE muscle that a rapid disruption of sarcoplasmic components occurred, and some disorganization of the myofilaments occurred during the 25 hour postmortem chilling period.

Biochemistry. Forrest et al. (1968); Judge et al. (1968); Lister et al. (1970) and Sair et al. (1970) indicated that the stress-susceptible animal has a smaller amount of creatine phosphate (CP), adenosine triphosphate (ATP) and larger amounts of lactic acid than stress-resistant pigs. Stress-susceptible pigs had higher levels of creatine phosphokinase (CPK) in the blood and the enzyme levels were correlated with measures of meat quality.

High lactic acid and low CP levels at the time of exsanguination were associated with PSE characteristics in muscle. (Kastenschmidt et al. 1968; Lister et al. 1970).

Jones et al. (1976) studied fifteen Hampshire barrows weighing 88 to 93 Kg possessing both PSS and stress-resistant strains and found no significant differences for cardiac muscle levels of glucose-6-phosphate, CP, ATP or acetate in both strains. However, at exsanguination, hearts from stress-susceptible pigs tended toward higher ATP and lactate levels than hearts from stress-resistant animals.

Muscle fiber types. The development of PSE muscle is related to muscle fiber types (Briskey, 1964; Beecher et al. 1965b). Red muscle is much more resistant than white muscle to development of PSE musculature (Briskey, 1964). Dildey et al. (1970) worked with forty Hampshire-Yorkshire cross pigs and stated that high proportion of light to dark muscle fibers, large light fiber diameters and lack of tenderness are common to animals with PSE musculature as well as to those with muscular

carcasses. Quantities of muscle pigment were inversely related to muscularity. However, Lister et al. (1967) found larger dark fibers in the PSE musculature. This is disagrees with the finding of Dildey.

Howe et al. (1968) and Beecher et al. (1968) found PSE muscles have a higher light to dark fiber ratio than normal muscles presumably because of the anaerobic metabolism which is predominant in light fibers. Merkel (1971) observed three muscle fiber types and found a higher % white fibers ( $\alpha W$ ) and a lower % red fibers ( $\beta R$ ) in PSE muscle, but no difference in % intermediate fibers ( $\alpha R$ ). Ashmore et al. (1972b) suggested that an increase in muscularity is accompanied by a transition from  $\alpha R$  to  $\alpha W$  fibers.

Cooper et al. (1969) working with Poland China and Chester White pigs, found muscle from stress-susceptible animals had more intermediate ( $\alpha R$ ) fibers than did muscle from stress-resistant animals. Certain intermediate fibers from stress-susceptible animals had a high amylophosphorylase and ATPase activity. The number and nature of the intermediate ( $\alpha R$ ) fibers are key contributors to stress-susceptibility of the animal and to the development of PSE characteristics in the musculature.

Swatland and Cassens (1973) reported a higher % intermediate ( $\alpha R$ ) and/or white fibers ( $\alpha W$ ) in PSE muscle than in normal muscle. However, Sair et al. (1970) working with stress-susceptible pig muscle and de Bruin (1971) with PSE muscle

found a greater proportion of red fibers (includes intermediate fibers) compared to stress-resistant and normal pig muscle.

Giant fibers. Cooper et al. (1969) found a large number of giant fibers in muscles from all stress-susceptible animals, but none from stress-resistant animals. Giant fibers apparently occur in PSE muscle, but not all PSE muscle contains them. Cassens et al. (1969) reported that the giant fibers may be indicative of abnormal muscle which has been implicated in the problem of stress-susceptibility and PSE muscle. Linke (1972) found giant fibers in both PSE and normal pig muscle.

Hendricks et al. (1971) found that giant fibers were round, large and often located near the periphery of the fasciculus, but not in every fasciculus.

Cooper et al. (1969) reported that in porcine muscle, giant fibers have a variable reaction for diphosphopyridine nucleotide tetrazolium reductase (DPNH-TR), a very negative reaction for amylophosphorylase, and very positive reaction for ATPase. Hunt (1973) also reported that giant fibers were observed in four bovine muscles of four quality-morphology groups and were distinctly round, tended to exhibit more crenation, were usually  $\alpha$ W and NADH-TR negative. Occasionally the giant fibers stained  $\alpha$ R and NADH-TR positive or  $\alpha$ W and NADH-TR positive.

Dubowitz et al. (1960) reported that dystrophic muscle has a majority of abnormally larger fibers (100 to 200 um) which react weakly for all oxidative enzymes and strongly for phosphoylase.

de Bruin (1971) indicated that giant fibers were not found in pig biopsy tissue but were observed in samples of a different muscle removed postmortem. The giant fiber might be a phenomenon induced postmortem.

Dutson et al. (1978) reported that giant fibers were observed in approximately one-third of the muscle samples, but in all instances comprised less than 1% of the total myofiber population. The usual ultrastructural banding pattern of normal myofibers was completely absent in giant myofibers. Giant myofibers had no recognizable mitochondria or sarcoplasmic reticulum but contained more vacuolar structures than normal fibers and giant fibers showed nuclear proliferation in some areas.

Capillary : fiber ratio. Romanul (1965) reported greater vascular density and/or greater blood flow to red muscle or red fiber areas than to white muscles or white fiber areas. Cooper et al. (1969) found no difference in capillary fiber ratio between normal (from stress-resistant pigs) and PSE (from stress-susceptible pigs) muscle. Cooper et al. (1969), Dilley et al. (1970) and Merkel (1971) indicated PSE pig muscle had a lower number of capillaries per unit muscle area even though the capillary : fiber ratio did not significantly differ from normal muscle.

Melton (1971) found capillaries per unit area of bovine muscle to be related to sire effects and several palatability and carcass traits.

Meat quality and palatability. Kauffman et al. (1964) reported that dry, firm, dark (DFD) ham muscle with a relatively high pH, shrank less during curing and cooking and was more juicy and tender than pale, soft, exudative (PSE) muscle tissue with low pH. Ham muscle acidity was not related to flavor.

Lewis et al. (1963) and Dildey et al. (1970) reported that firm pork chops with high pH are more tender and show less cooking loss than soft pork chops with low pH. But Judge et al. (1958, 1960) disagreed in that broiled pork chops from DFD loins were less tender than from PSE loins.

Carpenter (1961) and Kauffman (1964) reported no difference in tenderness or flavor in a comparison of light vs. dark colored pork loins, but a significantly higher cooking loss in the light colored loins, and a significantly higher juiciness score for the dark colored loins.

Briskey (1963) studied exhaustive exercise and sucrose feeding immediately prior to slaughter. Pigs on a high sucrose ration had pale, soft, watery muscles which were less tender and had greater cooking losses than controls. Pigs that were exercised had firmer, darker, more tender muscles and lowest cooking losses. Pale, watery ham from sucrose fed pigs were also soft, exudative and pale after curing and smoking.

Sayre and Briskey (1963) reported that PSE muscle had lower water holding capacities and protein solubility properties. Meat from PSE pork absorbed more curing pickle which penetrated

more rapidly into the muscle compared to normal meat.

Topel et al. (1976) studied pork loins from carcasses weighing 68 to 75 Kg. Pale chops had a significantly higher cooking loss than normal or dark colored chops. Consumer panels scored pale chops significantly lower in organoleptic acceptability than normal or dark chops. A trained panel gave a similar results. When the consumer panel selected pork chops from a retail display case, the normal colored chops received the highest rating and PSE chops the lowest. The pale chops were the most unstable in appearance and developed a greenish-gray cast after 2 to 3 days storage. The normal colored chops had significantly more intramuscular fat and less protein than either pale or dark chops.

Hedrick et al. (1963) reported that ham and loin muscles were firmer ( $P < .01$ ) and darker from carcasses that contained more marbling in the longissimus. Firmer and darker loin chops from Hampshire barrows had higher shear force, while softer and lighter colored chops from Durocs had higher shear force. Firmer chops from both breeds had lower cooking losses.

Skelley et al. (1971) found age, carcass length, rib eye area, specific gravity and % loin are negatively related to marbling of the longissimus. Backfat thickness was positively related to both carcass firmness and fat iodine number. Carcass firmness was negatively related to carcass length and specific gravity. Color of the longissimus muscle showed no significant relationship to any other factors studied. Ham muscle color

was negatively related to weight, carcass cooler shrink and positively related to the % shoulder. Barrow carcasses had more backfat, a smaller longissimus muscle, and a smaller % ham than gilt carcasses. Selection of swine for low backfat and a high % lean should not result in a reduction of palatability of the cooked chops.

Hedrick and Kauffman (1972) indicated that PSE muscle is more acidic (especially during early stages postmortem) and will shrink more, losing a proportionally greater volume of juices that contain vitamins (thiamin, riboflavin, niacin) and protein. It is pale, soft and loose textured. Within a carcass, some muscles (longissimus, gluteus medius) exhibit PSE chops which when either baked or broiled shrink nearly twice that of normal or DFD chops. PSE muscle appears to be more tender but somewhat drier when baked.

Berry et al. (1970) indicated that pork lean quality attributes (lean color score, lean firmness score, amount of marbling) were generally affected by age only between 200 and 240 days of age.

David (1975) studied marbling, color, firmness and muscle structure which are generally accepted quality indicators for predicting palatability attributes (flavor, juiciness, tenderness and over all satisfaction). He concluded that:

1. Marbling in pork chops is more highly associated with juiciness than with either tenderness or flavor.
2. Dark-colored pork usually sustains lower cooking

losses, possesses superior water holding capacity and receives higher taste panel ratings for juiciness, than light colored pork.

3. Increased firmness in pork muscle is generally associated with darker lean color, more fat and marbling, lower moisture content, greater water binding capacity and higher shear force requirement.
4. Low scores for muscle structure are associated with an increased incidence of pale, soft watery pork which generally has lower palatability ratings than normal pork.

Pale, Soft and Exudative (PSE)  
and Dark, Firm and Dry (DFD) Beef

Occurrence. Mackintosh and Hall (1935) concluded that dark-cutting beef was the result of some undetermined factor affecting the condition of "muscle hemoglobin" rather than quantity of hemoglobin present. Lawrie (1958) and Hedrick *et al.* (1959) have conclusively linked the occurrence of dark-cutting beef (DCB) with stress. Exercise and nutritional stress alone were not sufficient to induce DCB consistently, but imposed together increased the incidence of this condition (Howard and Lawrie, 1956).

Lawrie (1958) showed that at higher pH values the mitochondrial enzyme cytochrome oxidase was more active than

at lower pH values, and speculated that increased oxygen consumption of "dark-cutting" meat could increase the concentration of deoxygenated myoglobin and result in dark color.

Hedrick et al. (1959) reported that DCB can occur far more consistently in the bovine as a result of "psychic stress", presumably mediated through the release of epinephrine. In DCB, epinephrine acts on muscle tissue to activate a limiting enzyme, glycogen phosphorylase, thereby accelerating glycogen metabolism (Ashmore et al. 1971b). Under sustained stress, the muscle glycogen stores can be depleted. If the carcass is depleted of glycogen at slaughter, muscle with a high ultimate (48 hr) pH results, since postmortem anaerobic metabolism of glycogen is responsible for attaining the lower pH normally observed. Hall et al. (1944) reported a positive relationship between high muscle pH and the incidence of "dark-cutting" beef.

Meat quality. Loeffel (1942) indicated that dark-cutting beef was more tender than bright beef. Bright cutting samples were rated more desirable in flavor than dark-cutting (DC) samples.

Hunt et al. (1973) studied the histochemical and histological characteristics of bovine muscles from four groups: (1) normal in color, firmness and exudate (NOR), (2) normal in color but soft and exudative (NSE), (3) pale in color, soft and exudative (PSE) and (4) dark in color, firm and dry (DC). They found an increase in %  $\alpha$ R fibers and a decrease in % both  $\beta$ R and  $\alpha$ W fibers in NSE, PSE and DC groups. The loin steaks

from DC group were more tender than steaks from the other three quality groups. Warner-Bratzler shear values were lower for steaks from DC and PSE groups. No significant differences were detected for juiciness or flavor of steaks between quality groups. They also indicated that incidence of the PSE condition in beef appears to be less than 1% but the NSE condition is relatively common. There were no significant differences among the groups in % moisture and ether extract.

Valin (1971) indicated that the higher pigment levels of beef, compared with pork, tend to prevent manifestation of the pale, soft, exudative condition.

Mitochondria. Ashmore et al. (1972c) isolated mitochondria from normal and DCB carcasses and found that mitochondrial oxygen consumption is rapidly inhibited by the low pH environment of normal carcasses following slaughter, whereas capacity for oxygen consumption by mitochondria from "dark-cutters" continues for at least 5 days after death.

Prevention. Hedrick et al. (1959) used tranquilizer (thorazine) and insulin in attempts to counteract effects of epinephrine, but these were not successful. Ashmore et al. (1973) suggested that the use of a  $\alpha$ -adrenergic blockade agent might be successful in counteracting the epinephrine effects.

## Muscle Fiber Diameter

Introduction. Mammalian and avian skeletal muscle cells are long, unbranched, threadlike fibers which taper slightly at both ends. They vary considerably in diameter, ranging from 10 um to more than 100 um, within the same species, and even within the same muscle. Fiber length ranges from 1 to 40 mm (Hamm, 1965). In some muscles the fibers may run in a longitudinal direction (parallel to the long axis of the muscle), but in a muscle like the longissimus muscle, fibers are arranged at an angle to the long axis of the muscle (Eisenhut et al. 1965).

Muscle fiber diameter varies with species, muscle, postnatal development in body weight, chronological age, general body size, nutrition, activity, breed and fiber type.

Tenderness. Tenderness is the most important of the palatability factors in beef and has been attributed to many characteristics, including fiber diameter. Brady (1937) worked with four beef muscles (triceps brachii, longissimus, adductor and semitendinosus) and obtained a non-significant correlation between fiber diameter and cooked shear force. Tuma et al. (1962) working with twenty-three Herefords of five different age groups which ranged from six to ninety months old, found an increase in fiber diameter with increasing age in longissimus muscles. When age effects were not removed, the larger the fiber diameter, the greater the shear force (less

tender) and the lower the panel tenderness score. But when age effects were removed, fiber diameter was not related to shear force or panel score.

Romans et al. (1965) reported that beef carcass maturity had been considered to be an important factor influencing the palatability of meat. When they compared A, B, C and D maturity and moderate versus slight marbling, neither maturity nor marbling had a significant effect on tenderness. Moderately marbled steaks were significantly more juicy and had significantly larger muscle fiber diameters than steaks with slight marbling. More mature carcasses tended to have larger muscle fiber diameters than youthful carcasses. Tenderness was not significantly correlated with fiber diameter.

Hiner et al. (1953) removed samples from various beef wholesale cuts from cattle ranging in age from two months to five and one half years. They indicated that neck and foreshank muscle fibers were largest in diameter, followed by round and chuck muscles (muscles unidentified). The tenderloin muscle was smallest in diameter. Shear force estimates of tenderness and fiber diameter were positively related for all muscles studied and ranged from  $r=.31$  in foreshank muscles to  $r=.75$  in neck muscles. He indicated that meat having small fibers is more tender than meat having large fibers. Locker (1960) also reported that fiber diameter in bovine muscle is inversely related to tenderness and sarcomere length. Herring et al. (1965) found high correlations between bovine fiber diameters and tenderness

(.73), or sarcomere length and tenderness (-.80), and a high correlation (-.82) between fiber diameter and sarcomere length. They stated that fiber diameter and sarcomere length were factors in tenderness but may or may not be major factors.

Carpenter *et al.* (1963) reported maximum fiber diameter of porcine longissimus dorsi muscle ranged from 30 to 86 um. The correlation coefficient between fiber diameter and raw shear (-.20), cooked shear (.23), raw denture tenderometer value (-.38) and cocked denture tenderometer value (.45) may be explained by the fact that  $\frac{1}{2}$  inch cores taken from muscles of carcasses that had smaller muscle fibers, contained more fibers in the core. Therefore, more sarcolemma and endomysial connective tissue was present per core, resulting in a less tender product. Opposite results with the cooked samples may be explained either by selective alternation and subsequent tendering of the connective tissue in the cooked samples or by a major decrease in tenderness due to denaturation of the fibrillar proteins. With an increase in maximum muscle fiber diameter, there was a decrease in the taste-panel tenderness scores for cooked longissimus muscles.

Lewis *et al.* (1977), working with seventy-six steers, found low but significant correlations between tenderness and either fiber diameter or sarcomere length. The selection of animals for large fiber diameter (more muscling) may have small effects on tenderness of the muscle.

Meat quality. Miller *et al.* (1975) reported that porcine

quality (color-gross morphology score and marbling score), was unrelated to either fiber diameter or fiber number. Fast-growing pigs appeared to have more but smaller muscle fibers than slow-growing pigs.

Swanson (1965) reported a highly significant positive correlation between size of muscle fibers and Warner-Bratzler shear force values for bovine longissimus.

Size of fiber (diameter and area) is proportionate to size of the fiber bundle. Size of muscle bundles (fibers grouped by perimysial connective tissue) is positively associated with visible coarseness of a cross-sectional area of muscle and increases with chronological age of the animal. Texture of muscle may be assessed by estimating the apparent muscle bundle size (coarseness). Increases in thickness of connective tissue strands and size of muscle fibers and bundles may contribute to rough, coarse and undesirable texture appearing on the cut surface of muscle (Carpenter *et al.* 1963). They also indicated that muscle fiber diameter increased with age and was positively associated with thickness of connective tissue and bundle size. The relation between percent fat and fiber diameter was positive but not significant.

May (1975) studied twelve Simmental X Angus (SXA), twelve Hereford X Angus (HXA) and twelve Limousin X Angus (LXA) crossbred steers that were slaughtered in three equal groups (four from each breed) after 200, 242 and 284 days on feed after weaning. He reported that longissimus muscle fiber diameters between

breeds were not significantly different. Over all breeds, diameter of  $\beta$ R fibers was inversely related to indices of muscling and positively related to indices of quality (longissimus fat %, marbling and quality grade).

Meatiness. Blunn and Gregory (1935) and Smith (1963) found muscle fiber diameter was positively correlated with characteristics which express the meat content of animal such as loin eye area or to muscle mass in poultry. Joubert (1956) stated that a close relationship exists between muscle fiber diameter and total musculature in lamb. Staun (1963) found significant correlations between porcine fiber diameter and meatiness.

Livingston et al. (1966) working with longissimus muscle samples removed by biopsy from four pigs at intervals from weaning to 125 lbs. live weight, reported poor correlations between mean fiber diameter and "lean meat content".

Tuma et al. (1962) calculated correlation coefficients between total pounds of carcass lean and average fiber diameter to be .83 (L) and .73 (ST). When the animal age effect was removed, the correlations were 0.00 and 0.35 for longissimus and semitendinosus. They indicated that fiber diameter may not be a good indicator of total carcass lean or muscle size.

Joubert (1956) indicated a relationship between muscle fiber size (diameter and area) and body size.

Breed and Selection. Hendricks et al. (1971) evaluated

postmortem shortening, myoglobin content and muscle fiber diameter from 10 Poland China and 10 Hampshire pigs, and found that Poland China pigs generally had larger muscle fiber diameter and required less time to reach maximum shortening than muscle fibers from Hampshire pigs. Joubert (1956) indicated that fiber size was influenced by animal breed.

Staun (1963) studied diameter and numbers of fibers in longissimus dorsi in pigs of Danish Landrace, Pietrain, Duroc Jersey, Yorkshire, Veredeltes Landschwein and Deutsches Weisses Edelschwein and found that the diameter and number of fibers differed in pigs of different breeds. The diameter of fiber was influenced by age and weight of animals. No significant difference was found in number and diameter of muscle fibers from gilts, boars and castrates of the same breed when the animals were slaughtered at 90 Kg. The number and diameter of muscle fibers were different in different muscles from the same pig.

David et al. (1975) studied thirty-six steers which were divided equally between Angus and Charolais breed types. Cattle were slaughtered after 153 days (Group 1) and 253 days (Group 2) on a standard finishing ration. Group 2 steers had significantly larger  $\beta$ R fiber diameters and somewhat larger  $\beta$ R fiber areas than Group 1 steers. Breed effects showed Charolais steers had larger fiber diameters and areas than Angus for all fiber types.

Lewis et al. (1977) indicated that beef breed of sire

and dam affected fiber diameter and sarcomere length of the uncooked and cooked muscle, but had little effect on tenderness.

Smith (1963) recorded that selection of chickens for increased body weight resulted in increased fiber number and fiber size (diameter and area) in sartorius and pectineus. Selection of animals for increased body weight would not be expected to necessarily produce the same results as selection for high lean yield or for muscularity, since the increase of body weight would tend to include high fat deposition as a component. Increased body weight as a selection parameter could encourage extensive fat deposition rather than muscle tissue.

Hanrahan et al. (1973), using lines of mice that had been selected for high and low body weights, concluded that selection for body weight can have various effects on structure of individual muscles. In most cases large line mice had a greater fiber number, but not all had a significant increase in fiber size as well.

Nutrition. Berry et al. (1967) studied 18 Yorkshire barrows fed equal grain levels of 1) corn, 2) barley and 3) wheat. Average muscle fiber diameter for barley and wheat-fed hogs was significantly higher than corn-fed hogs. There were no significant differences among sample locations for muscle fiber diameter. Samples were taken from lateral, central and dorsal locations in the longissimus at the 10th rib. The correlation between average muscle fiber diameter and tenderness

was .25. Staun (1963) found insufficient quantities of protein in the ration to cause a decrease in muscle fiber diameters.

Muscle fiber types. Miller et al. (1975) studied 136 barrows and 131 gilts (Hampshire, Yorkshire and Hampshire X Yorkshire) and found that crossbred pigs had smaller muscle fibers than either parent line. Gilts had significantly larger fibers than barrows. Bundle diameter was positively related to fiber diameter and slightly negatively associated with measures of the proportion of dark fibers ( $\beta R$ ). An increase in diameter of any one of the 3 fiber types was accompanied by an increase in diameter of the other two fiber types. Ashmore et al. (1972b) indicated that heavier muscled animals are expected to have a larger proportion of large diameter fibers ( $\alpha W$ ).

Rigor Mortis. Melton et al. (1974) reported that variation in fiber diameter in beef is slightly less for those taken by biopsy with restraint of sample length than at four days postmortem which undergo variable fiber size changes associated with rigor mortis. Mean fiber diameters of biopsy ( $76.98 \pm 5.02$  um) and postmortem muscle ( $76.02 \pm 5.66$  um) were essentially the same. Hegarty et al. (1971) found that post-rigor tissues have smaller dimensions than pre-rigor tissue. He stated that extra-cellular fluid volume was larger in rigor muscle than pre-rigor muscle, and concluded that the movement of fluid from intra- to extra- cellular compartment should be related to water holding capacity.

Sampling. Swatland (1975) indicated that two major problems make it difficult to estimate the number of myofibers present in a whole muscle (real number). A transverse section through a muscle fasciculus will not include any myofibers which have terminated intrafascicularly before reaching the plane of sectioning (Swatland and Cassen, 1972). In longissimus muscle, arrangement of fasciculi makes it impossible to transect all fasciculi in any one transverse section of the muscle (Swatland, 1975). Thus, the number of myofibers appearing in a muscle transverse section (apparent number) may be considerably less than the real number.

For beef muscle samples removed from three different locations (dorsal, middle, lateral) in longissimus muscles at the 10th rib, fiber diameter decreased from the dorsal to lateral position (Tuma et al. 1962).

Swanson (1965) studied ribs and short loins which were removed 24 hours postmortem from right and left sides of five good grade beef carcasses. Samples were taken from 5 anterior to posterior positions along the LD and 5 locations within each position. He found that the smallest fibers were found over the 12th rib, and fibers increased in size both anteriorly and posteriorly to this region. The largest fibers were found between the last thoracic and first lumbar vertebral locations in the short loin region. The number of fibers per unit area indicates that smallest fibers are found at the lateral edge of the longissimus dorsi over the 12th rib. A great deal of

variation existed in muscle fiber size both among different positions along the longissimus muscle and among different locations within each position. The studies pertaining to muscle fiber diameter or meat texture should carefully consider sampling procedures. Large and significant differences in longissimus muscle fiber size were found among animals of the same weight and grade.

Alsmeyer et al. (1965) used the slice tenderness evaluator (STE) to test the tenderness of longissimus muscle of pork and beef. They found that the beef STE shear values at the dorsal location were significantly lower (more tender) than those from medial or lateral locations. However, STE puncture readings at the lateral position in pork were lower than those at the medial position.

Cagle et al. (1970) worked with 10 market-weight Hampshire pigs to test two methods (hot slice and cold slice) of fabricating pork loins and found fiber diameter was significantly affected by method of slicing. Larger fiber diameter was found for cold-slice muscle (76.4 vs. 73.6 um). Longissimus dorsi sliced before removal of body heat (hot slice) had significantly higher shear value (less tender) than cold sliced after 24 hours chill (12.33 vs. 9.77 Kg).

Methods. Tuma et al. (1962) obtained small samples from each core which were fixed in 10% formalin in physiological saline solution (0.9% NaCl). A section from each core was placed in the Waring-Blendor with enough physiological saline

the blendor blades. The blades were reversed to avoid cutting fibers and the blendor ran 30 seconds at a reduced rate to tease fibers for measuring. A small portion of teased fibers and solution was poured into a coplin jar lid. Fifty straight fibers were measured from each core using a compound microscope with a 10X objective lens and a 10X eye piece containing a calibrated micrometer. One hundred and fifty fibers from each longissimus sample and 50 fibers from each semitendinosus sample were measured. All fiber diameter measurements are in um.

Hendricks et al. (1971) and Miller et al. (1975) used a photomicrographic technique to measure fiber diameter. Miller et al. (1975) randomly selected photomicrographs of one small and one large muscle bundle. A micrometer was placed directly under the histological slide when the photograph was taken allowing the exact magnification of every print to be measured (approximately X185). Each fiber in each of the 534 bundles was classified into one of three categories depending on its staining properties (by Sudan Black-B staining). A random sample of 10 fibers from each of the three types of staining patterns was measured with a TGZ3 Model 500 Zeiss Particle Size Analyzer to obtain the diameter and cross-sectional area. Fiber diameter was defined as the average cross-sectional dimension of a muscle fiber.

May (1975) used a "pickett circle master", a grid containing 45 individual diameters in the range of the fiber sizes,

to measure fiber diameters on cross-sectional photographs of muscle. To determine the locations of measurement, he first divided the circle (8.9 cm radius) into halves with a line drawn through the center of the circle and then further divided into quarters with a line drawn through the center of the circle at right angles to the first line. The "+" was placed inside the circle so its four points were placed in an area dense with fibers. The "+" could be rotated in any manner within the circle to obtain an optimum position. A circle with a 2.54 cm radius was then drawn at the end of each of the four points of "+" with the circle's central point lying on the four lines of the "+" 2.54 cm from the outer perimeter of the large (8.9 cm radius) circle. Fiber diameter was measured on any fiber which had at least half of its area inside any of the four small (2.54 cm radius) circles. The circles on the grid were moved over the individual fibers until a "best fit" was obtained.

Heritability Estimates  
and Correlation Coefficients of Carcass Quality

Introduction. The rapid rise in the standard of living and the change in dietary habits during the past two decades have greatly increased demand for lean meat. To meet this demand, intensive selection for less fat in swine carcasses has been conducted. The breeding goal has become a pig which

yields maximum lean and minimum fat. As selection has progressed toward this goal, it has become clear that meat quality also must be taken into account.

Heritability estimates represent an attempt to define statistically the proportion of total phenotypic variance that may be attributed to additive gene action. In general, estimates of heritability based on paternal half sib analyses are larger than those obtained from parent-offspring regressions. The latter, being less subject to bias, might be considered more realistic. Differences in estimation procedures may account in part for the wide variation in estimates of heritability for various performance traits.

Heritability estimates. Zoellner et al. (1963), Hetzer and Harvey (1967) and Gray et al. (1968) reported that selection was effective in reducing backfat. In addition, selection has been successful in separating lines on the basis of efficiency (Dickerson, 1947) and growth (Krider et al. 1946). Berruecos et al. (1970) evaluated effectiveness of selection for reduced backfat thickness and estimated heritability of the backfat probe measurement by developing a selection line and maintaining a control line from a crossbred population based on Minnesota No. 1, Tamworth and Duroc hogs. He reported a reduction in backfat of .65 cm per generation. Realized heritability for backfat thickness was .27, which is smaller than the .83 reported by Zoellner et al. (1963), but is in general agreement with those reported by Hetzer and Harvey (1967), (.46) and

Gray et al. (1968), (.32).

Hetzer et al. (1967) selected for both high and low backfat thickness through ten generations in two Duroc lines and through eight generations in two Yorkshire lines. An unselected control line derived from the same sources as the selected line was maintained in each breed. After ten generations of selection the high and low fat-Duroc lines differed by 2.6 cm or 68% of the initial mean. The difference between the two selected Yorkshire lines after eight generations was 1.4 cm or 44% of the initial mean.

Stanislaw et al. (1967) studied purebred pigs (Duroc, Hampshire, Beltsville No. 1) and those from crossbred litters. Heritability estimates within the purebreds were  $.03 \pm .06$ ,  $.28 \pm .06$ ,  $.55 \pm .12$  for 56-day weight, average daily gain and probed backfat, respectively. Corresponding estimates within the crossbreds were  $.19 \pm .09$ ,  $.39 \pm .10$  and  $.47 \pm .13$ .

Jensen et al. (1967) collected information over a period of 2 years from 585 pigs representing 268 dams and 116 sires and found that backfat thickness, area of longissimus dorsi, % lean cuts, ether extract and total moisture (apparently of longissimus), expressible juice and taste panel scores for fatness and flavor were highly heritable ( $h^2 = 0.40$ ) and firmness, color, meat film area, shear value and softness score were moderately heritable ( $h^2 = 0.20$  to  $0.40$ ). Low heritability estimates were obtained for pH, subjective weeping score, marbling and juiciness. Genetic correlations indicated that effective

selection for lower backfat thickness, and/or increased area of longissimus dorsi and % lean cuts would yield meat with a lower water holding capacity, lower intramuscular fat content, higher shear force value and lower scores for juiciness and flavor.

Siers et al. (1972) obtained data from 3,439 purebreds (Duroc, Hampshire, Poland China, Landrace, Yorkshire) and calculated heritabilities for loin eye length, loin eye depth, loin eye area, ham and loin %, 154-day weight, weaning weight and backfat to be .60, .60, .70, .35, .25, .15 and .25, respectively. Genetic correlations showed carcass backfat was negatively correlated with carcass length, loin eye area, loin eye length, loin eye depth and ham and loin %.

Aberle et al. (1971) worked with 369 Duroc barrows and gilts produced by random mating over four generations and indicated estimated heritability and standard errors for color score ( $.49 \pm .23$ ), marbling score by Wisconsin System ( $.02 \pm .20$ ), % reflectance at 525 nm ( $.49 \pm .26$ ), carcass length ( $.23 \pm .23$ ), loin eye area ( $.60 \pm .25$ ), backfat thickness ( $.17 \pm .21$ ), protein solubility (transmission value of Hart,  $.37 \pm .20$ ), and shear value ( $.40 \pm .11$ ). Muscle quality as measured by color score, % reflectance and protein solubility are moderately heritable and selection should effect rather rapid change.

Omtvedt (1968) indicated heritability of backfat thickness, loin eye area, firmness score, marbling score, ether extract, total moisture, shear value to be .53, .47, .30, .28, .42, .52 and .33, respectively. Enfield et al. (1961) found carcass

length, backfat thickness and loin eye area in swine are moderately heritable in the range of .40 to .60. Jonsson (1965) reported heritability in meat color score for castrated males (.60) and females (.32). Muscle color in pigs is moderately heritable and selection for muscle color would result in a genetic change in the average muscle color score. Allen et al. (1966) also studied the heritability of muscle color and other quality characteristics and found that selection can influence these traits.

Heritability estimates for fat content of the longissimus (marbling score and ether extract) ranged from .18 to 1.0 (Allen et al. 1966; Omtvedt, 1968; Jensen et al. 1967).

Aberle et al. (1971) reported the heritability estimates of color score, marbling score, carcass length, loin eye area and backfat thickness to  $.49 \pm .23$ ,  $.02 \pm .20$ ,  $.23 \pm .23$ ,  $.06 \pm .25$  and  $.17 \pm .21$ , respectively.

Correlation coefficients. Saffle and Bratzler (1959) reported that % ether extract of longissimus was highly correlated to backfat (.41). Batcher and Dawson (1960) reported high correlations between backfat thickness and marbling score. However, Carpenter et al. (1965) found low non-significant correlations between backfat thickness and marbling core. Skelley and Handlin (1971) found backfat was highly related ( $P < .01$ ) to carcass firmness. Jensen et al. (1967) observed positive associations between carcass backfat and muscle color measurements. The thicker the backfat is,

the darker the muscle color is. Omtvedt (1968) reported estimates of near zero.

Judge et al. (1960) reported correlations of -.30, -.25 and -.16 for color with tenderness, juiciness and flavor scores, respectively. Marbling related to these three factors showed correlations of -.70, .40 and .13. Firmness related to these three factors showed correlations of -.55, -.06 and -.12.

Skelley et al. (1973), working with pork carcasses, found that ham color showed a significant and positive correlation ( $P < .01$ ) with both marbling and color of the longissimus. Carcass quality factors (carcass firmness, longissimus muscle marbling, longissimus muscle color, ham color) were not significantly related to palatability factors (flavor, juiciness, tenderness and over all preference).

Correlation analysis showed that higher color-structure score is associated with low % reflectance and transmission value (-.63 and -.39, respectively) (Aberle et al. 1971). Shear value was slightly related to color and marbling scores and to transmission value (-.15, -.21, -.17, respectively). Shear value also was related to loin eye area and backfat thickness (-.28, -.17, respectively). They indicated that as muscling increases and backfat decrease, tenderness tended to decrease.

Wax et al. (1975), working on 115 purebred barrows of six breeds (Chester White, Duroc, Hampshire, Poland China,

Spotted Polands and Yorkshire), found no significant difference between breeds in serum CPK. Spotted Polands, Poland Chinas, Yorkshires and Hampshires received the lowest scores for firmness and marbling. Loin eye area was significantly and negatively correlated with scores for color, firmness and marbling and with % transmission.

Skelley et al. (1973) found a significant correlation (-.91) between percent moisture and percent ether extract in longissimus. The chemical analyses (% ether extract and % moisture) were not significantly related to Warner-Bratzler shear force.

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## CHAPTER III

MUSCLING SELECTION IN SWINE AND ITS EFFECTS  
ON MUSCLE FIBER TYPES AND FIBER DIAMETER

## SUMMARY

A "select" line of Duroc pigs (selection index based equally on more loin-eye area and less backfat determined by An/Scan) was developed. A "control" line obtained by random selection from the same base population was also maintained.

Twenty-four pigs in select line and sixteen in the control line from the fourth (1975) and fifth (1976) generations after selection was initiated were compared for longissimus (L) and red semitendinosus (RST) muscle fiber type percentages and fiber diameter, carcass quality scores (loin and ham color, marbling and firmness), loin-eye area, Warner-Bratzler shear force of longissimus, % ether extract, % moisture and % total, drip and volatile cooking losses.

Ham color and firmness % total, drip and volatile cooking losses were essentially the same for select and control lines. Those traits were not affected by our selection system through five generations, since quality means were not visibly reduced in select line pigs. Slightly lower marbling scores ( $P < .07$ ) and less intramuscular fat ( $P < .01$ ) found in select line pigs

point to some reduction in marbling. If the selection had continued through more generations, the differences between lines in carcass quality scores might possibly become a problem.

The loin-eye area in the select line pigs was larger ( $P < .01$ ) than that in the control line by  $3.36 \text{ cm}^2$ .

Both in L and RST muscles, the mean %  $\beta$ R fibers were the same in the two lines. Select line pigs tend to have higher %  $\alpha$ W fibers and lower %  $\alpha$ R fibers (L and RST muscles) than controls.

Neither % fiber type nor fiber diameter were significantly correlated to loin-eye area nor Warner-Bratzler shear force. Greater loin firmness was correlated ( $P < .01$ ,  $r = .61$ ) with higher %  $\alpha$ R fibers. Ham firmness was not related to %  $\alpha$ R fibers but negatively associated ( $P < .05$ ,  $r = -.45$ ) with L fiber diameter.

L %  $\alpha$ R and  $\alpha$ W fibers were not correlated to any % cooking losses. Percentage of  $\beta$ R fiber was negatively correlated ( $P < .05$ ) with % total and drip cooking losses ( $P < .05$ ,  $r = .66$  and .61). Fiber diameter was positively correlated with % moisture ( $P < .01$ ), % total cooking loss ( $P < .01$ ) and % volatile cooking loss ( $P < .01$ ), but negatively correlated with % ether extract ( $P < .01$ ). Percentage of  $\alpha$ W fibers was negatively correlated ( $r = -.83$ ) with %  $\alpha$ R fibers in L which was an expected result because of reported transformation of  $\alpha$ R to  $\alpha$ W fibers. However, in RST muscle, %  $\alpha$ W fibers was negatively correlated ( $r = -.85$ ) with %  $\beta$ R fibers.

Color of ham and loin muscles was correlated ( $P < .01$ ) with firmness of ham and loin muscles. Loin-eye area was positively correlated with Warner-Bratzler shear force ( $P < .01$ ), % total cooking loss ( $P < .05$ ) and % volatile cooking loss ( $P < .05$ ). The Warner-Bratzler shear force was not highly correlated to % fiber types, fiber diameter, % total, drip and volatile cooking losses.

L muscle fiber diameter in the select line was larger ( $P < .01$ ) than in the control line. RST muscle fiber diameters were not significantly different between select and control lines. Fiber diameters of RST muscle were larger ( $P < .01$ ) than for L muscle.

Giant fibers with  $\alpha W$  staining properties were found both in porcine L and RST muscles.

## INTRODUCTION

Selection of heavily muscled animals for breeding stock may increase carcass "quantity" but may also decrease carcass "quality" by increasing pale, soft, exudative (PSE) or dark, firm, dry (DFD) pork (Topel, 1969; Barton, 1972).

Reports have shown that, in general, the white ( $\alpha W$ ) or intermediate ( $\alpha R$ ) fibers of porcine muscle are related to the occurrence of the PSE condition (Briskey, 1964; Cooper *et al.* 1969; Dildey *et al.* 1970; Merkel, 1971). PSE muscle showed increased cooking loss (Carpenter, 1961; Kauffman *et al.* 1964; Hedrick and Kauffman, 1972), reduced tenderness (Sayre *et al.*

1964; Kauffman et al. 1964) and increased processing losses (Kauffman et al. 1964). However, Judge et al. (1958, 1960); Hedrick et al. (1963) and Merkel (1971) reported that firmer and darker pork chops were less tender than PSE or normal pork chops.

Staun (1963) reported that animals possessing large muscling fibers are often rapid growing and more muscular. Muscle fibers with large diameters were associated with decreased tenderness (Locker, 1960; Cassens, 1966; Herring, 1968).

Low pork quality may become an industry problem and there is a real need for more insight concerning individual muscle response to selection for muscling and how this response relates to stress and to carcass quality.

In this study, a "select" line of Duroc hogs (selection index based on more loin-eye area and less backfat determined by An/Scan) was developed. A "control" line obtained by random selection from the same base population was also maintained.

The objectives of this study were to study the muscle fiber types and diameter of longissimus (L) and red semitendinosus (RST) muscles in "select" and "control" line barrows in the fourth and fifth generations after selection was initiated. The study also included an investigation of correlations among % fiber types and fiber diameter and of these traits to Warner-Bratzler shear force, carcass quality scores (loin color, loin marbling, loin firmness, ham color, ham marbling, ham firmness),

loin-eye area, chemical analyses of L (% ether extract, % moisture), and cooking losses (% total cooking loss, % drip cooking loss and % volatile cooking loss).

#### MATERIALS AND METHODS

Sample selection and location. Pigs in the base population of purebred Durocs were farrowed in May, 1971. Twenty gilts and four boars were randomly selected to form the control line. The select line was formed by using the 20 most desirable gilts and four boars from the remaining base population based on an index with maximum loin eye area and minimum backfat thickness (estimated by the An/Scan and adjusted to 100 Kg) receiving equal emphasis. Live animal backfat thickness was estimated at three locations; shoulder above the elbow, at 10th rib and hip above the stifle joint, all about 3.8 cm from the mid-line. Averages of the three measurements were adjusted on a .028 cm/Kg basis to a 100 Kg live weight. Live animal loin eye area was estimated (An/Scan) at the 10th rib and adjusted to a 100 Kg live weight. The adjustment was  $.213 \text{ cm}^2/\text{Kg}$  live weight. Adjusted age to 100 Kg live weight was obtained by adjusting age on a .91 Kg/day basis. One restriction was that the least desirable animals because of obvious structural unsoundness (up to 20 percent) would not be considered as potential breeding animals.

Each year breeding animals were farrowed in May, produced

litters the following May and were replaced after producing one litter causing the generation interval to be one year. Full-sib and half-sib matings were avoided to minimize inbreeding.

All barrows were of approximately the same age and were reared with identical environments and feeding regimes. They were slaughtered at an average of 100 Kg live weight. Animals were watered but not fed for about 16 hours prior to slaughter.

Histological methods. For histological and histochemical studies, samples were removed 24 to 48 hours postmortem from the center of the L anterior to the 10th rib and RST muscle (Figure 1). Care was taken to sample the same portion of each muscle in order to minimize sampling error.

Muscle cubes approximately 3 x 1 x 1 cm were cut for transverse sectioning. Samples were placed on wet cork, immersed in isopentane chilled with liquid nitrogen for 30 sec to 1 min. Cubes were removed from isopentane, sealed in "poly" bags and stored in an ultra cold freezer (-80 C) until sectioned with a cryostat. Frozen serial sections (12 um) were sectioned on the cryostat and mounted on cover slips. Sections were air dried for at least 30 min before staining for alkaline ATPase activity by the method of Padykula and Hermann (1955) as modified by Guth and Samaha (1969) and for SDH activity using the procedure of Nachlas et al. (1957) as modified by Barka (1963). The pH values for pre-incubation and incubation

Figure 1. (A) Location of red semitendinosus (ST) muscle from which histological sample was taken.

R: Red area (ST)

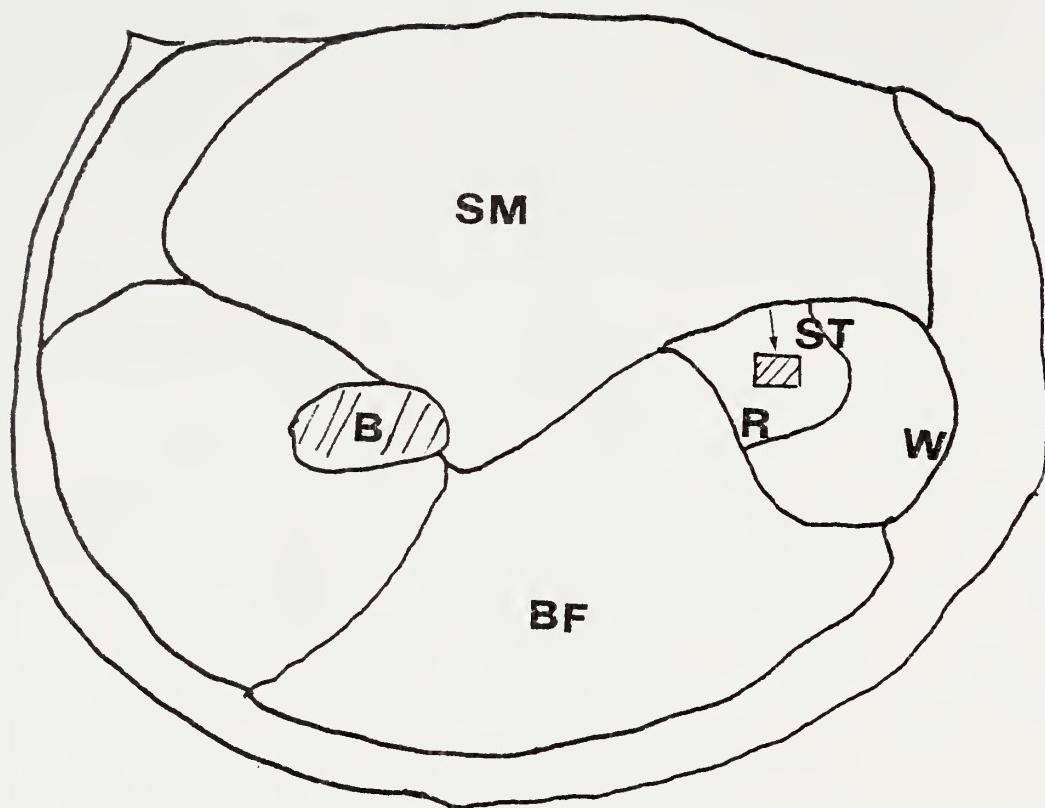
W: White area (ST)

B: Bone

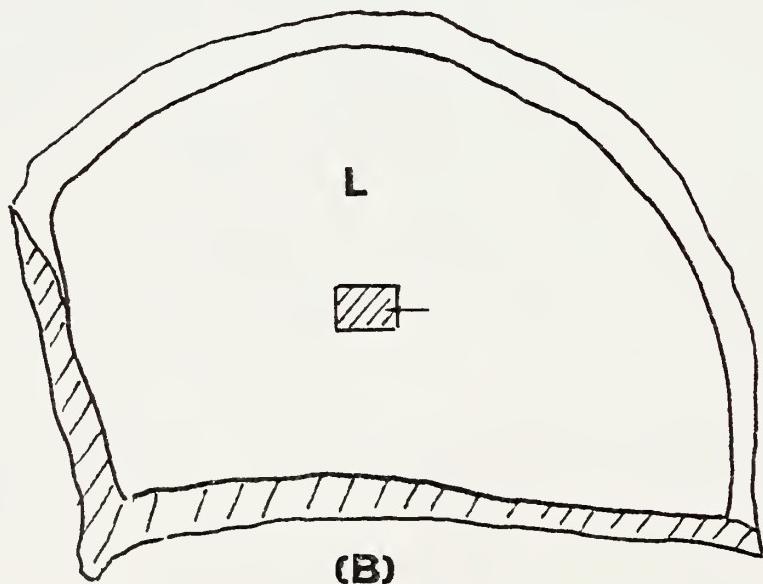
SM: Semimembranosus

BF: Biceps femoris

(B) Location of longissimus (L) muscle from which histological sample was taken.



(A)



(B)

of ATPase staining in L and RST are shown in Table 1 and their control is critical. Detailed staining procedures are outlined in Appendices A and B.

Fibers reacted for ATPase activity were classified according to the nomenclature of Ashmore & Doerr (1971a). SDH staining was used only as a check for correct fiber classification. Muscle fibers that stained positively (+) for both myosin ATPase and SDH activity were called  $\alpha$ R, fibers that stained strongly positive (++) with myosin ATPase but negative for SDH are  $\alpha$ W and fibers that do not react with myosin ATPase and are strongly positive (++) for SDH are  $\beta$ R.

Comparison of myosin ATPase and SDH stained serial sections (12um) of porcine L muscle and RST muscles are shown in Figure 2.

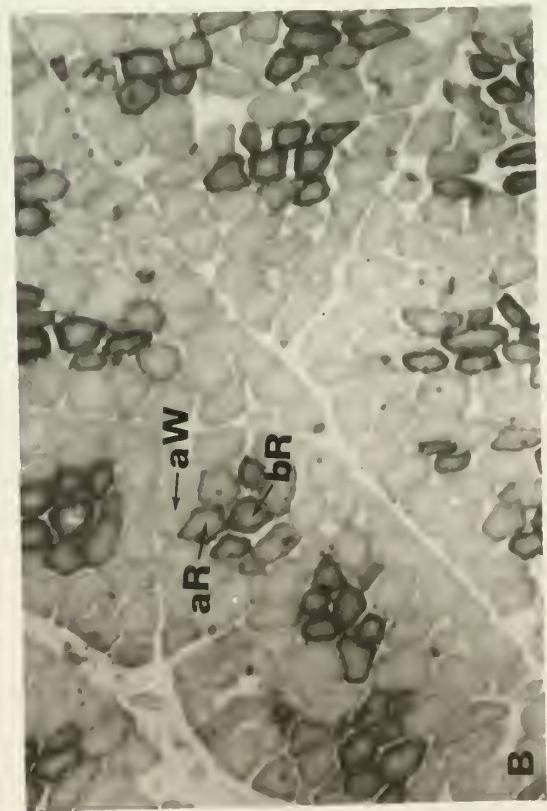
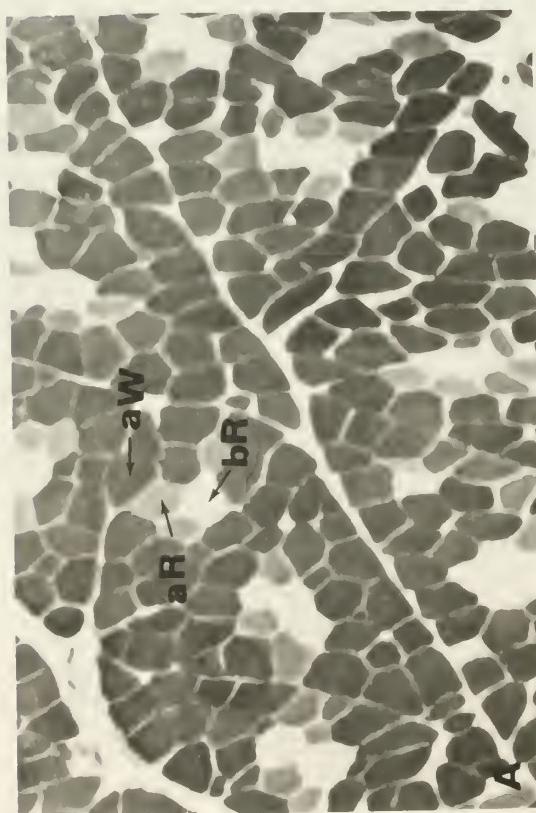
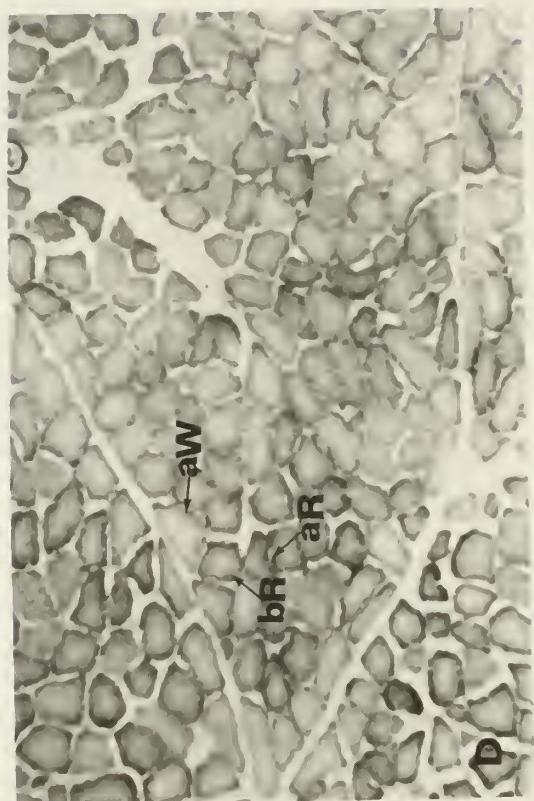
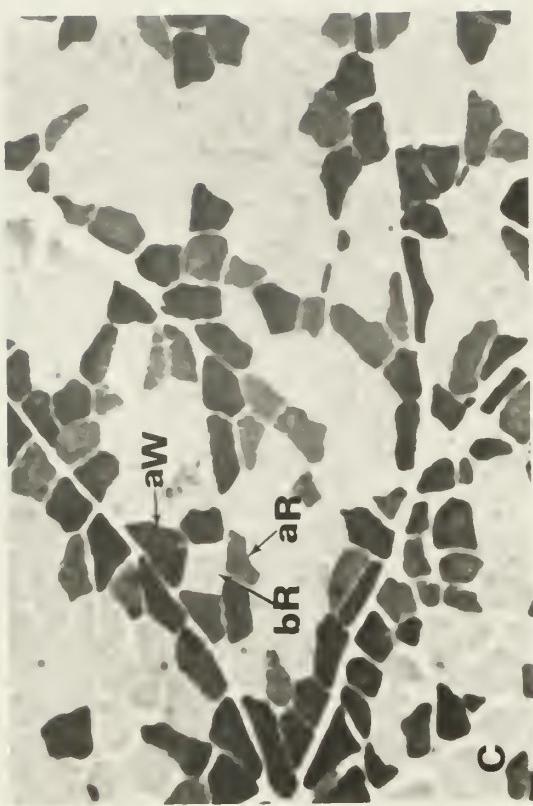
Photography. Photographs were taken with a Pentax Honeywell camera mounted on a Wild light microscope, using a 3X objective lens and 15X eyepiece. Three pairs (1 ATPase and 1 SDH at same location) of photographs, each pair at a different location, were used per muscle sample. Photographs were enlarged to 20.3 x 25.4 cm<sup>2</sup> and an area of approximate 100 fibers (10.2 x 15.2 cm<sup>2</sup>) was counted for determination of fiber types percentages.

Carcass measurements and quality scores. Carcass backfat thickness was the average of six measurements taken on the mid-line of both sides of the carcass, at the 1st rib, last rib and last lumbar. Carcass loin eye area at the 10th rib was traced and measured with a compensating polar planimeter. Color,

Table 1. pH VALUES FOR PRE-INCUBATION AND INCUBATION  
SOLUTIONS FOR MYOSIN ATPase STAINING IN  
L AND RST MUSCLES.

	Pre-incubation	Incubation
L	10.40 for 15 min	9.45 for 55 min
RST	10.45 for 15 min	9.35 for 60 min

- Figure 2. Comparison of myosin ATPase and SDH stained serial section (12  $\mu$ m) of porcine L muscle and RST muscles,  $\times 150$ .
- (A) Porcine L, ATPase.
  - (B) Serial section to (A), SDH.
  - (C) Porcine RST muscle, ATPase.
  - (D) Serial section to (C), SDH.



marbling and firmness scores were obtained for longissimus muscle at the 10th rib and at the muscle surface of the ham-loin junction. Color and firmness were separately evaluated on the basis of the Wisconsin Scoring System (1963). A score of 1 indicated extremely pale, soft and watery and a score of 5 represented very dark, firm and dry (See Appendix D). Marbling scores ranged from 1 (devoid<sup>-</sup>) to 36 (extremely abundant<sup>+</sup>), using beef marbling standards as a guide (See Appendix E).

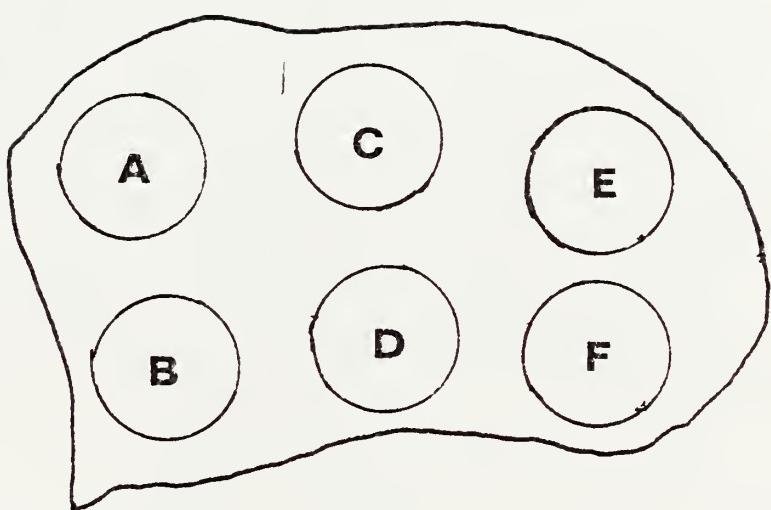
Chemical analysis of longissimus. Chemical analyses were determined on a ground sample of L at the 10th rib. Percent moisture and ether extract were determined by A.O.A.C. (1970) procedures.

Warner-Bratzler shear force and cooking losses. A 1-inch chop at 11th rib was used for Warner-Bratzler shear force determination and % total, drip and volatile cooking loss measurements. All the chops were frozen and stored at -29 C until evaluations were made. Chops used for shear force determinations were thawed to 5 C and trimmed of outside back-fat to .32 cm and modified roasted at 163 C to 73 C internally. Six 1.27 cm cores were removed by drill press coring device for shear force determinations for L muscle in the positions indicated in Figure 3. Six Warner-Bratzler shear values were averaged.

Fiber diameter. Muscle samples for measuring fiber diameter were put in a plastic bag with 4% neutral formalin and stored at 2 C until ready to use. Fiber diameters were

Figure 3. Cross section of the longissimus muscle  
(11th rib), illustrating the sample  
locations for Warner-Bratzler shear force.

- A: Medial-dorsal
- B: Medial-ventral
- C: Central-dorsal
- D: Central-ventral
- E: Lateral-dorsal
- F: Lateral-ventral



determined by the method of Tuma et al. (1962). A section from each core was placed in the Waring-Blendor with enough physiological saline (.9% NaCl) to cover the blender blades. The blades were reversed to avoid cutting fibers and the blender was run at low speed (50 rheostat) for 30 sec to tease the fibers apart. A small portion of teased fibers and solution was poured into a petri dish. Twenty five fibers were measured from each sample using a compound microscope with 10X objective lens and 15X eyepiece containing a calibrated micrometer. Wavy fibers were not included due to the inability to accurately measure their diameter. All fiber diameter measurements are in um (See Appendix C).

Statistical analysis. Data were subjected to a least squares analysis of variance. If F-test indicated significant difference for year and line interactions, mean pairs were tested by LSD (least significant difference,  $P < .05$ ). Pooled correlation coefficient analysis (within year and line) was used to ascertain the relationships among fiber type % and fiber diameter, carcass quality scores, loin-eye area, chemical analyses and cooking losses.

## RESULTS AND DISCUSSION

Table 2 presents the means of carcass quality scores, Warner-Bratzler shear force, loin-eye area, chemical analyses for L and cooking loss for two generations (1975, 1976) in

Table 2. MEANS OF CARCASS QUALITY SCORES, WARNER-BRATZLER SHEAR FORCE, LOIN-EYE AREA, CHEMICAL ANALYSES OF LONGISSIMUS (L) AND COOKING LOSSES IN SELECT AND CONTROL LINES (1975, 1976).

Carcass quality scores							
	Loin		Ham		Warner-Bratzler <sup>d</sup> shear force L		L
Year, Line	Color <sup>a</sup>	Marb. <sup>b</sup>	Firm. <sup>c</sup>	Color <sup>a</sup>	Marb. <sup>b</sup>	Firm. <sup>c</sup>	E.E. Moist. (%) Total Drip Volat.
Combined select	3.23	20.13	3.29	3.23	14.29	2.81	6.20
Combined control	3.25	23.50	3.16	3.25	16.75	3.00	5.72
Combined 1975	3.04	20.17	2.98	3.22	15.83	2.85	5.82
Combined 1976	3.50	23.24	*3.59	3.27	14.53	2.94	6.41
1975 Select	2.89 <sup>e</sup>	18.64	2.93 <sup>e</sup>	3.18	15.43	2.79	5.94
1975 Control	3.28 <sup>f</sup>	22.56	3.06 <sup>e</sup>	3.28	16.44	2.94	5.63
1976 Select	3.70 <sup>g</sup>	22.20	3.80 <sup>g</sup>	3.30	12.70	2.85	6.65
1976 Control	3.21 <sup>f</sup>	24.71	3.29 <sup>f</sup>	3.21	17.14	3.07	5.93

\*P < .05  
\*\*P < .01

<sup>a,c,l</sup> = extremely pale, soft and watery, . . . , 5 = very dark, firm and dry

<sup>b,l</sup> = devoid<sup>-</sup>, . . . , 36 = extremely abundant<sup>+</sup>.

<sup>d</sup>KG shear force, 1.27 cm core.

<sup>e,f,g</sup>Line x year means within column with same or superscript letters are not different (P < .05).

both select and control lines. Loin and ham color and firmness scores were not significantly different between select and control lines indicating that these characteristics did not become more pale, soft and exudative, with our selection for less fatback and more loin eye. However, Topel (1969) suggested that stress-susceptible pigs might produce pale, soft, exudative or dark, firm and dry musculature. A greater variability in color or firmness scores for select line carcasses would indicate more of both of these extremes. Loin color and firmness score for select line carcasses had slightly higher standard deviations, i.e. .68 and .62, compared to .45 and .35 for control line carcasses. Ham color and firmness and both loin and ham marbling score standard deviations were similar for both lines or larger for controls. Line x year interactions were calculated for loin color and firmness. Loin color was darker for select line carcasses in 1976, a reversal of line effects of 1975. Select loin scores in 1976 showed firmer, dryer muscles with no line effect noted in 1975. This could be an indication of more stress-susceptible pigs in the select line in the 5th generation which would follow from Pongchan's (1978) report of higher serum creatine phosphokinase (CPK) in select line pigs the same year.

The loin-eye area in the select line pigs was larger ( $P < .01$ ) than that in the control line by  $3.36 \text{ cm}^2$ . Pongchan (1978), working with the same population of Duroc pigs as our study, found that selection (maximum loin-eye area and minimum

backfat) reduced backfat thickness by 3.94% (.10 cm) in five generations compared with that of the control line. This value was less than the 16% decrease after five generations of selection reported by Zeller and Hetzer (1960) whose selection index was based only on less backfat. Bereskin and Darvey (1976) reported in Durocs, after 17 generations of selection (minimum backfat thickness), the select line pigs had larger loin-eye area by  $18.3 \text{ cm}^2$  and less backfat thickness by  $4.67 \text{ cm}^2$  than the control line, but these data were obtained after 12 more generations of selection than this study.

Slightly higher marbling scores ( $P < .07$ ) were found for both loin and ham muscles representing the control line. L in the select line had less ether extract % than the control line ( $P < .01$ ).

The Warner-Bratzler shear force was not significantly different between the select and control lines.

Percentage of total, drip and volatile cooking losses were essentially the same in the two lines. The slighter higher drip cooking loss for chops from the control line was likely due to higher ( $P < .01$ ) L ether extract. This agrees with Ashmore et al. (1972b) indicating that selection for heavy-muscled animals can result in a higher  $\alpha_2$ W fiber and less intramuscular and intracellular lipid. Topel (1969) and Barton (1972) reported that selection of heavily muscled animals for breeding stock decreased carcass quality and increased cooking losses.

A greater total cooking loss ( $P < .05$ ) for chops in 1976 compared to 1975 was largely due to a higher volatile cooking loss ( $P < .05$ ) which may have been partly due to a slightly higher moisture content. Drip cooking loss was lower ( $P < .05$ ) for 1976 samples. They also showed darker color and greater firmness scores. Year or seasonal effects could be related to climatic conditions. Judge et al. (1959) found pigs grown during the fall and winter had more highly marbled muscles.

The means of %  $\alpha$ R,  $\beta$ R and  $\alpha$ W fibers of porcine L and RST muscles in both select and control lines are shown in Table 3. Mean %  $\beta$ R fibers for both L and RST in select and control lines were essentially the same. Select line pigs tended to have higher  $\alpha$ W fiber and lower  $\alpha$ R fiber % in the L and RST controls, but differences were not significant. These observations tend to agree with Ashmore et al. (1972b) who suggested that selecting "more muscular farm animals" results in selecting for a higher %  $\alpha$ W fibers, because the  $\alpha$ R fibers have the capacity to transform to  $\alpha$ W fibers.

The %  $\beta$ R fibers in RST was higher ( $P < .05$ ) in 1976 than 1975. This was not expected as Ashmore et al. (1972b) indicated that  $\beta$ R fiber % remains relatively stable.

Five generations may not be enough to show major changes in fiber type, although trends are developing.

Hunt (1973) showed abnormal beef groups had high %  $\alpha$ R fibers. Merkel (1971) showed PSE pork had increased %  $\alpha$ W fibers

Table 3. MEANS OF PERCENTAGE OF  $\beta$ R,  $\alpha$ R and  $\alpha$ W FIBERS  
 OF PORCINE LONGISSIMUS (L) AND RED  
SEMITENDINOSUS (RST) MUSCLES IN SELECT  
 AND CONTROL LINES (1975, 1976).

Year, Line	Fiber types			n
	$\beta$ R (%)	$\alpha$ R (%)	$\alpha$ W (%)	
<b>L muscle</b>				
Combined select	8.54	19.80	71.69	20
Combined control	8.17	23.13	68.75	12
Combined 1975	8.31	21.91	69.80	20
Combined 1976	8.53	19.61	71.89	12
1975, Select	8.68	20.41	70.93	12
1975, Control	7.76	24.16	68.11	8
1976, Select	8.31	18.89	72.83	8
1976, Control	8.98	21.05	70.03	4
<b>RST muscle</b>				
Combined select	44.85	26.64	28.40	19
Combined control	46.56	27.69	25.75	14
Combined 1975	42.86	27.51	29.66	19
Combined 1976	49. <sup>*</sup> 26	26.51	24.04	14
1975, Select	41.82	25.39	32.83	11
1975, Control	44.29	30.43	25.30	8
1976, Select	49.01	28.35	22.31	8
1976, Control	49.60	24.05	26.35	6

\*P < .05

and decreased %  $\beta$ R fibers; the %  $\alpha$ R fibers was not different. Cooper et al. (1969) indicated stress-susceptible pigs had higher %  $\alpha$ R fibers. Swatland and Cassens (1973) found PSE pork had higher %  $\alpha$ W and/or  $\alpha$ R fibers.

Comparison of % muscle fiber types in L and RST muscles are shown in Table 4. L muscle contained 70.84%  $\alpha$ W fibers and 29.18% red fibers ( $\alpha$ R and  $\beta$ R). RST is a red muscle which contained 26.85%  $\alpha$ W fibers and 73.07% red fibers ( $\alpha$ R and  $\beta$ R). Beecher et al. (1965b) reported that porcine muscles that contain more than 40% red fibers could be called red muscles, white muscles contain less than 30% red fibers. By this definition the red area of ST can be classified as a red muscle and the L is clearly a white muscle.

Table 5 presents pooled correlation coefficients (calculated within line x year groups) among L muscle fiber types and fiber diameter and of these traits to carcass quality scores, Warner-Bratzler shear force, loin-eye area, chemical analyses and cooking losses. Color scores of loin and ham muscle were not highly correlated with %  $\alpha$ R,  $\beta$ R and  $\alpha$ W fibers nor with L fiber diameter. Marbling scores of loin and ham muscle were not strongly related to any fiber type % or to fiber diameter. Allen et al. (1966) reported no relationship between porcine fiber diameter and color score. Miller et al. (1975) found porcine fiber diameter was unrelated to color score and marbling score. Merkel (1971) found a higher %  $\alpha$ W fiber and a lower %  $\beta$ R fibers in PSE pork muscle. Swatland

Table 4. MEANS OF PERCENTAGE OF  $\beta$ R,  $\alpha$ R AND  $\alpha$ W  
 FIBERS OF PORCINE LONGISSIMUS (L)  
 AND RED SEMITENDINOSUS (RST) IN BOTH  
 GENERATIONS (1975, 1976).

	L	RST
Over all		
$\beta$ R (%) .	8.42	** 46.06
$\alpha$ R (%)	20.76	** 27.01
$\alpha$ W (%)	70.84	** 26.85
$\beta$ R + $\alpha$ R (%)	29.18	** 73.07
1975		
$\beta$ R (%)	8.31	** 42.86
$\alpha$ R (%)	21.91	** 27.51
$\alpha$ W (%)	69.80	** 29.66
1976		
$\beta$ R (%)	8.53	** 49.26
$\alpha$ R (%)	19.61	** 26.51
$\alpha$ W (%)	71.89	** 24.04

\*\*P < .01

Table 5. POLLED CORRELATION COEFFICIENTS AMONG HISTOLOGICAL CHARACTERISTICS OF LONGISSIMUS (L) AND TO CARCASS QUALITY SCORES, WARNER-BRATZLER SHEAR FORCE, LOIN-EYE AREA, CHEMICAL ANALYSES OF LONGISSIMUS, AND COOKING LOSSES.

Item	L			
	$\beta R$ (%)	$\alpha R$ (%)	$\alpha W$ (%)	Fiber diameter
<u>Carcass quality scores</u>				
Loin color <sup>a</sup>	-.39	.40	-.19	.26
Loin marbling <sup>b</sup>	-.17	.11	-.02	-.14
Loin firmness <sup>c</sup>	-.39	.61**	-.40	.80
Ham color <sup>a</sup>	-.30	-.19	.37	-.22
Ham marbling <sup>b</sup>	.22	-.23	.12	-.43
Ham firmness <sup>c</sup>	.12	-.17	.11	-.45*
<u>Loin-eye area</u>	-.33	.22	-.04	.27
<u>Warner-Bratzler shear<sup>d</sup> (L)</u>	-.29	.04	.14	.15
<u>Chemical analyses (L)</u>				
Ether extract (%)	.16	-.15	.06	-.60**
Moisture (%)	-.15	.18	-.10	.55**
<u>Cooking loss, %</u>				
Total	-.49*	.41	-.14	.66**
Drip	-.48*	.09	.20	.30
Volatile	-.36	.41	-.21	.61
<u>Histological characteristics</u>				
$\alpha R$ (%) (L)	-.34			
$\alpha W$ (%) (L)	-.24	-.83**		
Fiber diameter (L)	-.44	.42	-.18	

\*P < .05

\*\*P < .01

<sup>a</sup>c<sub>1</sub> = extremely pale, soft and watery..., 5 = very dark, firm and dry.

<sup>b</sup>c<sub>1</sub> = devoid<sup>-</sup>, ..., 36 = extremely abundant<sup>+</sup>.

<sup>d</sup>Kg shear force, 1.27 cm core.

and Cassens (1973) also reported a higher %  $\alpha R$  and/or  $\alpha W$  fibers in PSE muscle than normal muscle. Hunt (1973) reported that bovine L and gluteus medius muscles which were normal in color but soft and exudative had the highest %  $\alpha R$  fibers, followed by pale, soft and exudative muscles and dark-cutting muscles. The lowest %  $\alpha R$  was noted in the normal color, firmness and exudation groups. Differences in %  $\beta R$  and  $\alpha W$  fibers between these quality groups were generally non-significant.

Loin firmness was correlated ( $r=.61$ ,  $P < .01$ ) with %  $\alpha R$  fibers. Ham firmness was not related to %  $\alpha R$  fibers but was negatively associated ( $P < .05$ ) with fiber diameter.

The % various fiber types and of fiber diameter were not significantly correlated to loin-eye area or Warner-Bratzler shear force. Melton et al. (1975) indicated a low correlation between bovine fiber types and Warner-Bratzler shear force. Tuma et al. (1962) reported that, when age effects were removed, bovine fiber diameter was not significantly related to L muscle area. Correlations of .31 and .49 between loin-eye area and porcine fiber diameter were reported by Livingston et al. (1966) and Staun (1968).

Melton et al. (1974) found a correlation of .28 between loin-eye area and bovine L muscle fiber diameter in beef. Our study showed a correlation between loin-eye area and fiber diameter of .27. Brady (1937); Tuma et al. (1962) and Melton et al. (1974) did not obtain significant relationships between bovine fiber diameter and shear force. Hiner et al. (1953)

and Herring et al. (1965) found a high positive correlation between bovine fiber diameter and shear force. Muscles having large fiber diameters had higher shear force values than muscle having smaller fiber diameters.

Our study showed low correlation of L % ether extract and % moisture with %  $\beta$ R, %  $\alpha$ R and %  $\alpha$ W muscle fibers. Melton et al. (1975) reported very low correlations of % ether extract and % moisture with %  $\beta$ R and  $\alpha$  fibers ( $\alpha$ R and  $\alpha$ W) for bovine L muscle.

Fiber diameter was negatively correlated ( $P < .01$ ,  $r = -.60$ ) with % ether extract (wet basis) and positively correlated ( $P < .01$ ,  $r = .55$ ) with % moisture. Carpenter et al. (1963) reported no significant correlation ( $r = .24$ ) between porcine fiber diameter and % ether extract (moisture-free basis). Melton et al. (1974) reported the correlation of .35 and -.18 for bovine fiber diameter with % ether extract and % moisture, respectively.

Percent  $\alpha$ R and  $\alpha$ W fibers was not correlated to any cooking loss %.  $\beta$ R fiber % was negatively correlated ( $P < .05$ ) with % total cooking loss ( $r = -.49$ ) and % drip loss ( $P < .05$ ,  $r = -.48$ ). Fiber diameter was positively correlated with % total cooking loss ( $P < .01$ ,  $r = .66$ ) and % volatile loss ( $P < .01$ ,  $r = .61$ ), partly due to the association of larger fiber diameter with high % moisture.

Percent  $\alpha$ W fibers was negatively correlated ( $P < .01$ ,  $r = -.83$ ) with %  $\alpha$ R fibers. Ashmore et al. (1972b) suggested that  $\alpha$ R fibers have the capacity to transform to  $\alpha$ W fibers and this

high negative correlation would support his suggestion.

The low negative relationship of fiber diameter to %  $\alpha W$  fibers ( $r=-.18$ ) was not expected since these fibers are generally larger in diameter and a higher %  $\alpha W$  should be related to larger fiber diameters for such muscles. The negative association of %  $\beta R$  with fiber diameter was expected because these fibers are generally smaller in diameter, but this correlation is lower than expected. Perhaps these weak and unexpected relationships suggest sampling problems for the fiber diameter method, in which fibers in physiological saline (.9 % NaCl) solution are teased apart.

Pooled correlation coefficients among histological characteristics of RST and of these traits to carcass quality scores, Warner-Bratzler shear force, loin-eye area, chemical analyses and cooking loss are shown in Table 6. Percent fiber types and fiber diameter of porcine RST were not related to any of the carcass quality scores or to Warner-Bratzler shear force, loin-eye area, chemical analyses and cooking losses of L samples. These characteristics of the RST are poor predictors of composition and cooking losses of another muscle, namely the L.

The only significant correlation was of %  $\alpha W$  fibers ( $P < .01$ ,  $r = -.85$ ) with %  $\beta R$  fibers. This differs from L and perhaps indicates less variation in transformation between  $\alpha R$  and  $\alpha W$ , so that animal (carcass) differences in  $\alpha W$  are strongly reflected by differences in %  $\beta R$ . Transformation occurs between

Table 6. POOLED CORRELATION COEFFICIENTS AMONG HISTOLOGICAL CHARACTERISTICS OF RED SEMITENDINOSUS (RST) AND OF THESE TRAITS TO CARCASS QUALITY SCORES, WARNER-BRATZLER SHEAR FORCE, LOIN-EYE AREA, CHEMICAL ANALYSES AND COOKING LOSSES.

Item	RST			
	SR (%)	αR (%)	αW (%)	Fiber diameter
<u>Carcass quality scores</u>				
Loin color <sup>a</sup>	.30	-.07	-.28	.00
Loin marbling <sup>b</sup>	.18	-.21	-.07	.24
Loin firmness <sup>c</sup>	.27	-.05	-.25	.19
Ham color <sup>a</sup>	.25	.02	-.28	-.16
Ham marbling <sup>b</sup>	.05	-.34	.14	.30
Ham firmness <sup>c</sup>	.39	-.32	-.24	.23
<u>Loin-eye area</u>	.05	-.13	.02	.21
<u>Warner-Bratzler shear<sup>d</sup> (L)</u>	.43	-.30	-.28	-.04
<u>Chemical analyses (L)</u>				
Ether extract (%)	.13	-.23	.00	.41
Moisture (%)	-.13	.15	.05	-.38
<u>Cooking loss, %</u>				
Total	-.26	-.08	.32	.17
Drip	-.01	.01	.00	-.15
Volatile	-.27	-.09	.34	.23
<u>Histological characteristics</u>				
αR (%) RST	-.36			
αW (%) RST	-.85**	-.18		
Fiber diameter RST	-.23	-.05	.27	

\*\*P < .01

<sup>a</sup>c<sub>1</sub> = extremely pale, soft and watery, ..., 5 = very dark, firm and dry.

<sup>b</sup>c<sub>1</sub> = devoid<sup>-</sup>, ... 36 = extremely abundant<sup>+</sup>.

<sup>d</sup>Kg shear force, 1.27 cm core.

$\alpha W$  and  $\alpha R$ , but %  $\beta R$  has been reported to be relatively stable by Ashmore et al. (1972b).

Table 7 shows the pooled correlation coefficients among carcass quality scores, Warner-Bratzler shear force values, chemical analyses and cooking losses. L color showed no significant relationship to any of the factors studied except loin firmness ( $P < .01$ ,  $r = .72$ ). Ham color was significantly correlated with ham firmness ( $P < .05$ ,  $r = .53$ ). Loin marbling score was associated with ham marbling score ( $P < .05$ ,  $r = .50$ ), and % L ether extract ( $P < .05$ ,  $r = .46$ ). Ham marbling score was correlated with % ether extract ( $P < .01$ ,  $r = .61$ ) and % moisture ( $P < .01$ ,  $r = -.59$ ) of the L. Henry and Bratzler (1960) and Skelley and Handlin (1971) reported little relationship between fat content of pork muscle and its color. Our study observed the same result, but Hedrick et al. (1968) found that hams and loins were firmer ( $P < .01$ ) and darker in color from carcasses that contained more marbling in the L.

Shear force was not significantly correlated with any loin or ham color, marbling or firmness score. Skelley et al. (1973) reported low non-significant correlations between shear force and porcine carcass quality (L firmness, marbling, or color and ham color).

Loin-eye area showed a significant and positive correlation with Warner-Bratzler shear ( $P < .01$ ,  $r = .60$ ), % total cooking loss ( $P < .05$ ,  $r = .50$ ) and % volatile cooking loss ( $P < .01$ ,  $r = .58$ ).

Table 7. POOLED CORRELATION COEFFICIENTS AMONG CARCASS QUALITY SCORES, WARNER-BRATZLER SHEAR FORCE, LOIN-EYE AREA, CHEMICAL ANALYSES OF LONGITISSIMUS (L) AND COOKING LOSSES.

Item	Carcass quality scores						Warner-Bratzler shear force	Loin eye area	E.E. Moist. (%)	L	Cooking loss, % Total Drip
	Loin a Color	Loin b Marb.	Loin c Firm.	Ham a Color	Ham b Marb.	Ham c Firm.					
Carcass quality scores											
Loin marbling <sup>b</sup>	.34										
Loin firmness <sup>c</sup>	.72 **	.29									
Ham color <sup>a</sup>	.18	-.44 *	.19								
Ham marbling <sup>b</sup>	.00	.50 *	.04	-.28							
Ham firmness <sup>c</sup>	.06	-.15	.35	.53 *	.36						
Warner-Bratzler shear (L)	.07	.02	.22	.04	-.23	.06					
Loin-eye area	-.05	.12	.10	-.25	-.10	.19	.60 **				
Chemical analyses (L)											
E.E. (%)	-.16	.46 *	.08	-.25	.61 **	.34 *	-.03		.20		
Moisture	.16	-.20	-.06	.09	-.59 **	-.48 *	-.08		-.27	-.88 **	
Cooking loss, %											
Total	.27	-.16	.16	-.18	-.10	-.20	.29	.50 *	-.26	.18	
Drip	.27	-.11	.13	.45	.01	.10	-.20	-.11	-.50 *	.46 *	.37
Volatile	.20	-.13	.13	-.34	-.11	-.24	.38	.58 *	-.12	.04	.95 ** .06

\*P < .05  
\*\*P < .01

acl = extremely pale, soft and watery, ..., 5 = very dark, firm and dry.

b<sub>1</sub> = devoid-, ..., 36 = extremely abundant<sub>+</sub>.

d<sub>KG</sub> shear force, 1.27 cm core.

Drip loss % was negatively related to % ether extract ( $P < .05$ ,  $r = -.50$ ) and positively related to % moisture ( $P < .05$ ,  $r = .46$ ). This observation is difficult to explain since drip loss contains considerable lipid. However, it also contains protein and moisture. Total % cooking loss was highly correlated with % volatile loss. The Warner-Bratzler shear force was not significantly related to % total cooking loss, % drip cooking loss and % volatile cooking loss. Sayre et al. (1964) found muscles with lower cooking losses were more tender, whereas muscles with higher cooking loss had higher Warner-Bratzler shear force.

Means of porcine muscle fiber diameter and fiber numbers per unit area of L and RST are presented in Table 8.

L muscle fiber diameter in the select line was larger ( $P < .01$ ) than in the control line. RST muscle fiber diameter in select line was larger than in the control line, but the difference was not significant. Smith (1963) found that selection for body size of chicks would result in increased fiber size (diameter and area). Fiber diameters of RST muscle were larger ( $P < .01$ ) than the fiber diameters in L muscle.

The method used is subject to overestimation of some fibers, particularly those which vary widely from round cross-sectional shape. The results are also extremely dependant on which fibers are selected for fiber diameter measurement. Eisenhut et al. (1965) indicated that the mammalian and avian skeletal muscle fiber diameter ranged from 10 um to more than

Table 8. FIBER DIAMETER (um) AND FIBER NUMBERS  
 OF PORCINE LONGISSIMUS (L) AND RED  
SEMITENDINOSUS (RST) MUSCLES IN SELECT  
 AND CONTROL LINES (1975, 1976).

	<u>Fiber diameter</u>		<u>Fiber number per unit area</u>	
	<u>L</u>	<u>RST</u>	<u>L</u>	<u>RST</u>
Combined select	87.13	**	99.34	
	**			
Combined control	79.56	**	97.06	
Combined 1975	89.25	**	105.67	
	**		**	
Combined 1976	78.23	**	90.64	
1975, Select	92.29	**	108.37	125.20
1975, Control	86.21	**	102.98	145.50
1976, Select	81.96	**	90.30	139.60
1976, Control	72.90	**	91.14	137.10

\*\*P<.01

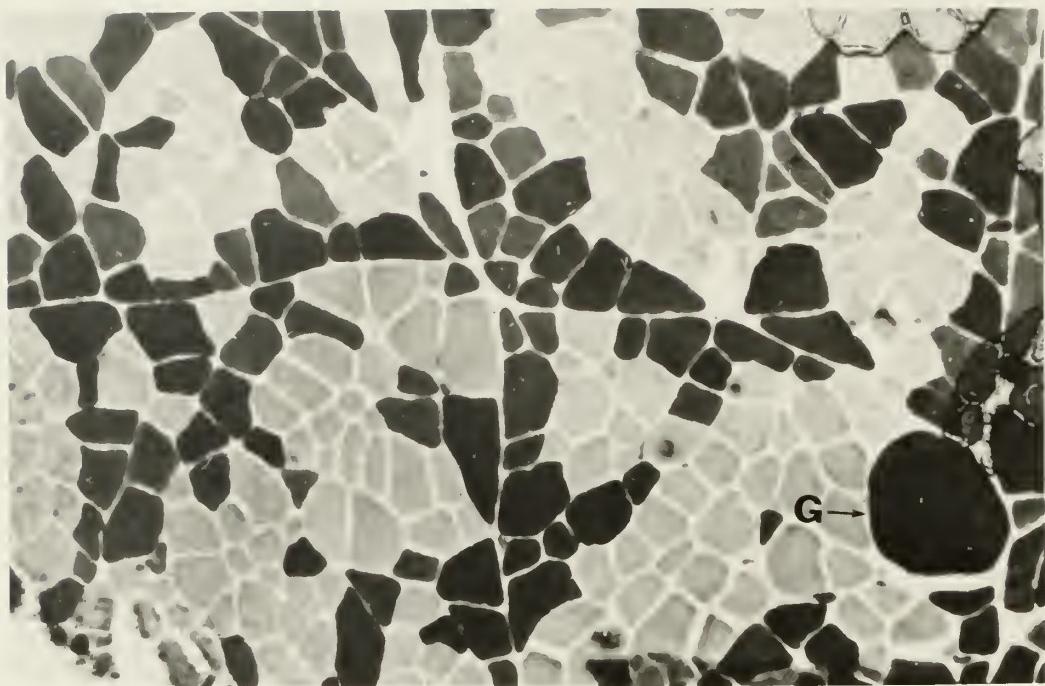
100 um within the same species, and even within the same muscle. Cagle and Henrickson (1970) found fiber diameter of porcine L muscle to average 76.4 um.

To check on results with the method of Tuma et al. (1962) fiber counts were taken on identical areas of photographs used for determination of fiber type. A higher number is expected for samples with smaller muscle fiber diameter.

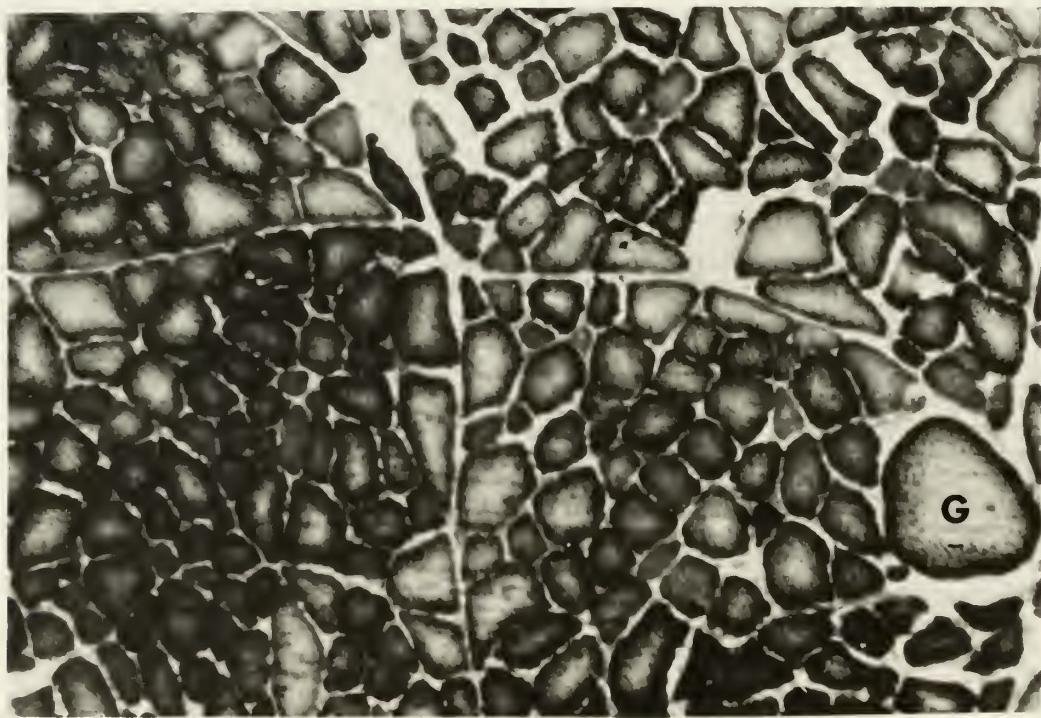
L had more fibers in a given area than RST ( $P < .01$ ), but lines were not different.

A year effect was noted ( $P < .01$ ) since larger fiber diameters were obtained from 1975. This might be due to small differences in technique such as selection of fibers for measurement. However, a year effect was not noted when mean fiber numbers in a given area of photographs used for fiber type were compared.

Figure 4 shows a giant fiber in alkaline myosin ATPase and SDH stained RST muscle. Cooper et al. (1969) found giant fibers to have a variable reaction for DPNH-TR, a very negative reaction for amylophosphorylase, and a highly positive reaction for ATPase. Hunt (1973) indicated that giant fibers were usually  $\alpha$ W and NADH-TR negative. Occasionally the giant fibers stained  $\alpha$ R and NADH-TR positive or  $\alpha$ W and NADH-TR positive. This study showed giant fibers to stain like  $\alpha$ W fibers and have a positive reaction for alkaline myosin ATPase and a negative reaction for SDH. Giant fibers were found both in porcine L and RST muscles.



A



B

Some researchers have suggested that giant fibers are an artifact and are due to drastic shortening of these muscle fibers. However, the cross-sectional area of the giant fiber in Figure 4 appears to be 5 or 6 times as large as the next largest  $\alpha W$  fibers. It seems highly unlikely that such drastic shortening could occur.

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## APPENDICES

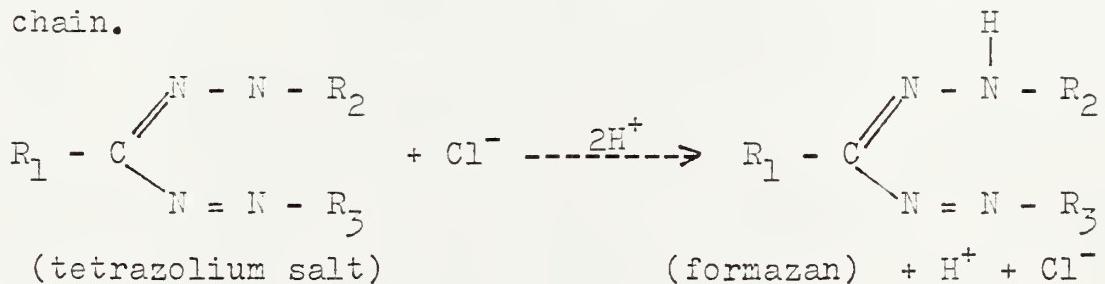
## APPENDIX A

## SUCCINIC DEHYDROGENASE (SDH)

(Nachlas et al. 1958 as cited by Barka et al. 1963)

## 1. Introduction:

Localization of dehydrogenase depends on reduction of a water-soluble tetrazolium salt to a water-insoluble formazan by one of the members of the biological oxidation chain.



The deposition of formazan in the tissue section will mark the sites of reduction.

Dehydrogenases represent the first step in biological oxidation of a variety of substances. SDH is linked directly with the cytochrome system.

SDH activity is demonstrated by incubating unfixed frozen sections with succinate in the presence of a convenient tetrazolium salt (Nitro BT) in a buffer medium. Enzyme activity is localized by a formazan pigment deposition and should be only in mitochondria. Non-specific reduction of tetrazolium salts does not occur to any significant extent under the specified conditions.

2. Reagents:

a. Incubating solution:

0.06 M sodium succinate (1.626%)	2.0	ml
0.2% Nitro blue tetrazolium (Nitro BT)	5.0	ml
0.2 M phosphate buffer (pH 7.4)	2.0	ml
Ringer solution (mammalian)	1.0	ml

pH of incubating solution is adjusted to 7.0 to 7.4.

Both sodium succinate and Nitro BT solutions can be stored at 2 to 4 C for months.

b. 0.2% Nitro BT (0.2g Nitro BT + 100 ml Distilled water)

c. Ringer solution(mammalian)

NaCl	0.9	g
KCl	0.03	g
CaCl <sub>2</sub>	0.03	g
H <sub>2</sub> O	100	ml

d. 10% formalin solution

40% formalin	10	ml
Distilled water	30	ml

e. Polyvinyl pyrrolidone (PVP) solution

Polyvinyl pyrrolidone	50	g
Distilled water	50	ml
(stand overnight)		

3. Procedure:

- a. Section frozen muscle (12 um) at -20 C and mount on coverslip.
- b. Bring mounted sections to room temperature and incubate without drying (less than 15 min).
- c. Incubate in incubating solution for 4 to 10 hours at 37 C.
- d. Rinse with cold distilled water for 3 min.

- e. Transfer to 10% formalin for 30 min.
- f. Rinse with distilled water for 3 min.
- g. Wash with 20 to 30% ethanol for 5 min.
- f. Rinse with distilled water for 3 min.
- i. Mount with PVP.
- j. Identify by species, muscle, stain, date, person-project.

## APPENDIX B

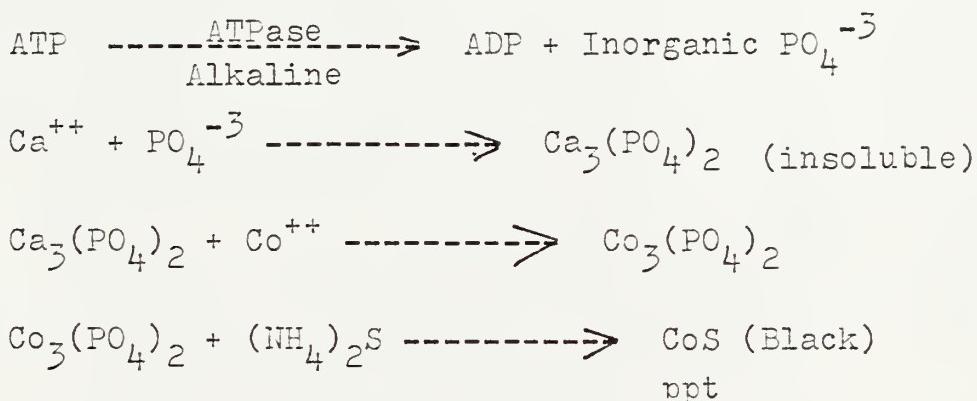
## ADENOSINE TRIPHOSPHATASE (ATPase)

In most mammalian muscles, three types of muscle fibers can be histochemically distinguished on the basis of qualitative differences in their actomyosin ATPase. The ATPase of the  $\alpha$ -fiber is acid-labile and base-stable, the ATPase of the  $\beta$ -fiber is base-labile and acid-stable, and the ATPase of the  $\alpha\beta$  fiber is intermediate in stability at both acidic and basic pH values. Acid pre-incubation causes white fibers to react negatively while alkaline pre-incubation causes white fibers to react positively. The pH of the pre-incubation medium is very critical. Species variability and muscle variability is found in pH lability of actomyosin ATPase.

The histochemical reaction depends upon a rather complex series of reactions. The tissue section is incubated in a solution containing ATP and calcium at a pH of 9.4. The enzyme ATPase splits off the terminal phosphate from ATPase, and because of the presence of calcium in the solution, this phosphate immediately combines to form calcium phosphate. At an alkaline pH, calcium phosphate is insoluble and is deposited at the site of enzyme activity. The tissue section is then removed from the solution and placed in a solution of cobalt chloride. Cobalt phosphate forms at the sites where calcium phosphate was previously present in the section. The tissue is

then placed in ammonium sulphide and black insoluble cobaltous sulphide forms. The site of the original enzyme activity is thus localized.

The ATPase present in various muscle fiber types seems to be particularly dependent upon the influence of pH. The pH effect can be used to demonstrate different fiber types by pre-incubating the tissue section at various pH values.



### 1. Reagents:

#### a. Guth's solution:

Formaldehyde solution (40%)	50	ml
Na Cacodylate (MW160)	31	g
CaCl <sub>2</sub> (MW147)	10	g
Sucrose (MW342)	115	g

Bring the final volume to 1 liter with water chilled to 4°C.

#### b. Alkaline pre-incubation solution:

Sigma No. 221 (2-Amino-2 methyl-1-propanol solution) buffer (1.5M)	3.35 ml
CaCl <sub>2</sub> (0.18M)	5.00 ml
Distilled water	40.00 ml

Adjust pH to 10.4 or 10.45 with KOH (1 to 10 N) using pH meter, and bring final volume to 50 ml with distilled water. This solution should be prepared fresh each use.

## c. Incubation solution:

Sigma No. 221 buffer (1.5M)	3.35 ml
CaCl <sub>2</sub> (0.18M)	5.00 ml
ATP, disodium (MW551.2)	76.00 mg
Distilled water	38.00 ml

Adjust pH to 9.35 or 9.40 with 6N HCL (using pH meter) and bring final volume to 50 ml with distilled water. This solution should be freshly prepared for each use.

d. 1% CaCl<sub>2</sub>:

CaCl <sub>2</sub>	1	g
Distilled water	100	ml

## e. 2% Cobaltous chloride:

CoCl <sub>2</sub>	2	g
Distilled water	100	ml

## f. 1% Yellow ammonium sulfate:

Ammonium sulfate	0.25	ml
Distilled water	25	ml

## 2. Procedure:

- a. Section frozen muscle (12 um) at -20 C in cryostate.
- b. Mount on coverslips and air dry at room temperature for 30 min.
- c. Fix 5 min in Guth's fixative at pH 7.6 (pH is critical).
- d. Rinse gently with distilled water for 1 min. Continuously change water.
- e. Pre-incubate at 37 C for 15 min with alkaline pre-incubation solution at pH 10.45 (semitendinosus muscle, ST), 10.40 (longissimus muscle, L).
- f. Rinse gently with distilled water for 2 mon.
- g. Incubate for 55 to 65 min at 37 C in incubation

solution at pH 9.35 (ST), 9.45 (L).

- h. Wash in three 30 sec changes of 1%  $\text{CaCl}_2$ .
- i. Place in 2%  $\text{CoCl}_2$  for 3 min.
- j. Wash in four 30 sec changes of distilled water.
- k. Place in 1% yellow ammonium sulfate for 3 min  
(use hood).
- l. Wash in several changes of tap water for 4 min.
- m. Dehydrate 3 min each in 80%, 95%, 95%, 100% and 100%  
ethyl alcohol.
- n. Clear 3 min each in xylene twice.
- o. Mount with permount.
- p. Identify by species, muscle, stain, date person-  
project.

## APPENDIX C

FIBER DIAMETER (Tuma et al. 1962)

## 1. Reagents:

## a. 4% Neutral Formalin

40% formalin	10	ml
Distilled water	90	ml
Na <sub>2</sub> HPO <sub>4</sub>	0.65	gm
NaH <sub>2</sub> PO <sub>4</sub>	0.49	gm
	100	ml

## b. Physiological Saline (0.9% NaCl)

Distilled water	1000	ml
NaCl	9	gm
	1000	ml

## 2. Procedures:

- a. Fix the samples in 4% neutral formalin for at least 48 hours (stored in cooler).
- b. Slice 1/8" thick sections from the sample.
- c. Place sections in Waring-Blendor (blades reversed). Add enough physiological saline just to cover the blades (about 50 ml).
- d. Blend at slow speed (50 rheostat) for 30 sec to tease fibers apart.
- e. Pour part of contents into petri dish and observe under low power of objective lens (10X) and filar micrometer (15X).
- f. Measure the diameter (width) of 25 different fibers. Position crosshair on one side take a reading, reposition crosshair to other side and read again.

## APPENDIX D

## VISUAL COLOR AND FIRMNESS SCORES\*

Visual Color Scores

1. Extremely pale
2. Pale
3. Grayish pink
4. Moderately dark
5. Dark

Visual Firmness Scores

1. Extremely soft and watery
2. Moderately soft and dry
3. Moderately firm and dry
4. Firm and dry
5. Very firm and dry

\*Wisconsin Special Bulletin #9, 1963.

## APPENDIX E

## DEGREE OF MARBLING SCORE

36	Extremely abundant	(EA <sup>+</sup> )	18	Modest	(Mt <sup>+</sup> )
35	Extremely abundant	(EA)	17	Modest	(Mt)
34	Extremely abundant	(EA <sup>-</sup> )	16	Modest	(Mt <sup>-</sup> )
33	Very abundant	(VA <sup>+</sup> )	15	Small	(Sm <sup>+</sup> )
32	Very abundant	(VA)	14	Small	(Sm)
31	Very abundant	(VA <sup>-</sup> )	13	Small	(Sm <sup>-</sup> )
30	Abundant	(A <sup>+</sup> )	12	Slight	(Sl <sup>+</sup> )
29	Abundant	(A)	11	Slight	(Sl)
28	Abundant	(A <sup>-</sup> )	10	Slight	(Sl <sup>-</sup> )
27	Moderately abundant	(MA <sup>+</sup> )	9	Trace	(Tr <sup>+</sup> )
26	Moderately abundant	(MA)	8	Trace	(Tr)
25	Moderately abundant	(MA <sup>-</sup> )	7	Trace	(Tr <sup>-</sup> )
24	Slightly abundant	(SA <sup>+</sup> )	6	Practical devoid	(PD <sup>+</sup> )
23	Slightly abundant	(SA)	5	Practical devoid	(PD)
22	Slightly abundant	(SA <sup>-</sup> )	4	Practical devoid	(PD <sup>-</sup> )
21	Moderate	(Me <sup>+</sup> )	3	Devoid	(D <sup>+</sup> )
20	Moderate	(Me)	2	Devoid	(D)
19	Moderate	(Me <sup>-</sup> )	1	Devoid	(D <sup>-</sup> )

## APPENDIX F

PROBABILITY OF YEAR, LINE AND YEAR X LINE EFFECTS FOR CARCASS QUALITY SCORES, WARNER-BRATZLER SHEAR FORCE, LOIN-EYE AREA, CHEMICAL ANALYSES OF LONGISSIMUS (L), COOKING LOSS AND HISTOLOGICAL CHARACTERISTICS.

Item	Y (Year)	L (Line)	YxL (Year x Line)
Carcass quality			
Loin color	.034*	.767	.014*
Loin marbling <sup>b</sup>	.107	.071	.688
Loin firmness <sup>c</sup>	.000**	.160	.023*
Ham color <sup>a</sup>	.820	.958	.468
Ham marbling <sup>b</sup>	.489	.068	.246
Ham firmness	.471	.156	.812
Loin-eye area	.879	.007**	.130
Warner-Bratzler shear <sup>d</sup> (L)	.365	.355	.702
Chemical analyses (L)			
Ether extract (%)	.279	.005**	.222
Moisture (%)	.011*	.144	.318
Cooking loss, %			
Total	.000**	.359	.923
Drip	.025*	.252	.703
Volatile	.000**	.552	.812
Histological characteristics			
$\alpha R$ (%) RST	.462	.874	.051
$\beta R$ (%) RST	.076	.656	.784
$\alpha W$ (%) RST	.160	.599	.089
$\alpha R$ (%) L	.292	.181	.715
$\beta R$ (%) L	.727	.915	.513
$\alpha W$ (%) L	.343	.167	.998
Fiber diameter (RST)	.000**	.432	.295
Fiber diameter (L)	.000**	.010**	.267

\*P < .05

\*\*P < .01

<sup>a,c</sup> 1 = extremely pale, soft and watery, ..., 5 = very dark, firm and dry.

<sup>b</sup> 1 = devoid<sup>-</sup>, ..., 36 = extremely abundant<sup>+</sup>.

<sup>d</sup> Kg shear force, 1.27 cm core.

MUSCLING SELECTION IN SWINE AND ITS EFFECTS  
ON MUSCLE FIBER TYPES AND FIBER DIAMETER

by

JAMES CHUN-CHIN KUO

B.S., NATIONAL CHUNG-HSING UNIVERSITY (TAIWAN), 1973

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of

the requirements for the degree

MASTER OF SCIENCE

FOOD SCIENCE

Department of Animal Sciences and Industry

KANSAS STATE UNIVERSITY

Manhattan, Kansas

1978

## ABSTRACT

A "select" line of Duroc pigs (selection index based equally on more loin-eye area and less backfat determined by An/Scan) was developed. A "control" line obtained by random selection from the same base population was also maintained.

Twenty-four pigs in select line and sixteen in the control line from the fourth (1975) and fifth (1976) generations after selection was initiated were compared for longissimus (L) and red semitendinosus (RST) muscle fiber type percentages and fiber diameter, carcass quality scores (loin and ham color, marbling and firmness), loin-eye area, Warner-Bratzler shear force of longissimus, % ether extract, % moisture and % total, drip and volatile cooking losses.

Ham color and firmness, % total, drip and volatile cooking losses were essentially the same for select and control lines. Those traits were not affected by our selection system through five generations, since quality means were not visibly reduced in select line pigs. Slightly lower marbling scores ( $P < .07$ ) and less intramuscular fat ( $P < .01$ ) found in select line pigs point to some reduction in marbling. If the selection had continued through more generations, the differences between lines in carcass quality scores might possibly become a problem,

The loin-eye area in the select line pigs was larger ( $P < .01$ ) than that in the control line by  $3.36 \text{ cm}^2$ .

Both in L and RST muscles, the mean %  $\beta$ R fibers were

the same in the two lines. Select line pigs tend to have higher %  $\alpha$ W fibers and lower %  $\alpha$ R fibers (L and RST muscles) than controls.

Neither % fiber type nor fiber diameter were significantly correlated to loin-eye area nor Warner-Bratzler shear force. Greater loin firmness was correlated ( $P < .01$ ,  $r = .61$ ) with higher %  $\alpha$ R fibers. Ham firmness was not related to %  $\alpha$ R fibers but negatively associated ( $P < .05$ ,  $r = -.45$ ) with L fiber diameter.

L %  $\alpha$ R and  $\alpha$ W fibers were not correlated to any % cooking losses. Percentage of  $\beta$ R fiber was negatively correlated ( $P < .05$ ) with % total and drip cooking losses ( $P < .05$ ,  $r = .66$  and .61). Fiber diameter was positively correlated with % moisture ( $P < .01$ ), % total cooking loss ( $P < .01$ ) and % volatile cooking loss ( $P < .01$ ), but negatively correlated with % ether extract ( $P < .01$ ). Percentage of  $\alpha$ W fibers was negatively correlated ( $r = -.83$ ) with %  $\alpha$ R fibers in L which was an expected result because of reported transformation of  $\alpha$ R to  $\alpha$ W fibers. However, in RST muscle, %  $\alpha$ W fibers was negatively correlated ( $r = -.85$ ) with %  $\beta$ R fibers.

Color of ham and loin muscles was correlated ( $P < .01$ ) with firmness of ham and loin muscles. Loin-eye area was positively correlated with Warner-Bratzler shear force ( $P < .01$ ), % total cooking loss ( $P < .05$ ) and % volatile cooking loss ( $P < .05$ ). The Warner-Bratzler shear force was not highly correlated to % fiber types, fiber diameter, % total, drip

and volatile cooking losses.

L muscle fiber diameter in the select line was larger ( $P < .01$ ) than in the control line. RST muscle fiber diameters were not significantly different between select and control lines. Fiber diameters of RST muscle were larger ( $P < .01$ ) than for L muscle.

Giant fibers with  $\alpha W$  staining properties were found both in porcine L and RST muscles.

